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A STUDY ON THE COMBINED BELZER AND COLLINS TECHNIQUES OF KIDNEY PRESERVATION

by

© JOHN F. MILNER, M.D.

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

EXPERIMENTAL SURGERY

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

FALL, 1973

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled A STUDY OF THE COMBINED BELZER AND COLLINS TECHNIQUES OF KIDNEY PRESERVATION submitted by JOHN FREDERIC MILNER in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in EXPERIMENTAL SURGERY.

.....
Supervisor

.....

.....

Date September 25, 1973

"A likely impossibility is always preferable
to an unconvincing possibility."

- Aristotle 384-322 B.C.

To my wife Sylvia,

who has supported me emotionally and financially

throughout my residency,

and

To my parents,

who raised me to appreciate the stimulation and

rewards of an academic life.

ABSTRACT

Numerous workers have obtained good results in kidney preservation experiments designed to reproduce the work of Belzer and Collins. There is, however, little information in the literature to indicate the tolerance of kidneys to various combined periods of hypothermic preservation, and hypothermic, pulsatile perfusion. Regardless of the lack of published data, human kidneys are being subjected to combined preservation methods in kidney sharing programs. This study was designed to investigate whether hypothermic preservation preceding, and following, pulsatile perfusion preservation, has a significant effect on the immediate function of the transplanted kidney.

The dog was chosen as the experimental model, and an autotransplant experimental program was designed. The left kidney was removed on Day One, and subjected to twenty-four hours of preservation. On Day Two, this kidney was autotransplanted into the same animal, and an immediate contralateral nephrectomy was performed. No donor pretreatment with such things as intravenous fluid, heparinization, mannitol diuresis, or no-touch surgical technique was performed, to more closely simulate the cadaver kidney procurement situation in the University Hospital, human transplant program.

Seven different preservation groups were used, the animals being assigned to these in a random fashion. Group 1 animals were the surgical controls, with the kidneys being removed, flushed with Collins solution, and immediately autotransplanted. From Groups 2

through 7, the kidneys were subjected to twenty-four hours of preservation. In each successive group, the kidneys were preserved for a higher percentage of the twenty-four hours by Collins technique, until in Group 7, Collins preservation alone was used.

In the post-operative period, kidney function was followed by frequent serum creatinine and blood urea nitrogen determinations. At six weeks, all animals were considered long-term survivors, and were subjected to renal function studies (glomerular filtration rates and renal plasma flows). Some animals were subjected to renal angiography and some kidney specimens were studied by electron microscopy. Tissue of all kidneys was analyzed by light microscopy.

Thirteen dogs were lost because of technical failure. The most frequent cause of technical failure was the presence of double renal arteries in the donor kidney. After elimination of the technical failures, the poorest overall survival rate was achieved in Group 7, in which sixty-six percent of animals survived six weeks. In the rest of the groups, over ninety percent of the animals survived six weeks post-transplant. After the control group (Group 1), Group 2 animals with twenty-two of the twenty-four hours preservation being on the Belzer, had the lowest post-operative rise in creatinine and blood urea nitrogen. In Group 7, the rise in the creatinine and blood urea nitrogen was the highest. The creatinine and blood urea nitrogen rises for Groups 3 to 6, lay between those for Groups 2 and 7. A statistical analysis indicated that there was a progressively poorer quality of preservation, the higher the percentage of the twenty-four hours preservation that Collins technique was used. Although this relation-

ship between groups was still present at six weeks, the absolute differences between their creatinines and blood urea nitrogens was small. No significant differences were noted between groups by light and electron microscopy, or by renal function studies done at six weeks post-transplant.

In conclusion, this project has shown that Collins and Belzer kidney preservation techniques can be successfully combined. The best quality of preservation was found in those kidneys stored for the majority of the twenty-four hours on the Belzer apparatus. This was much less apparent at six weeks post-transplant than in the immediate post-operative period. This left the impression that if the animals had been followed for longer than six weeks after transplant, the differences between groups might have been even less distinguishable.

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CHAPTER I

A. INTRODUCTION

In the sixteen years since human kidney transplantation has been seriously studied, numerous preservation methods have been used, but only a few have been satisfactory for application to humans. The ideal method for organ storage is that it be inexpensive, reliably reproducible, free of contamination hazards, so simple that the possibility of technical error is almost non-existent, and capable of producing indefinite storage (Watkins *et al*, 1971). Storage methods have included various combinations of brief, intermittent, or continuous perfusion with hypothermia and either normobaria or hyperbaria. The advantages of organ preservation in cadaver organ transplantation have been listed by many authors and include time for prospective tissue typing, time to prepare the recipient, time to predict organ viability, time to procure organs from outlying hospitals, time to choose from expanded recipient pools, and time to investigate diseases of the donor (Olsson *et al*, 1969).

Most preservationists now agree that a proper preservation technique should include kidney perfusion. There are three authors however (Hendry *et al*, 1968; Weber *et al*, 1969; Groenewald *et al*, 1971) who have spoken against perfusion, arguing that more glomerular and vascular damage ensues than if perfusion is discarded altogether. Some of the perfusates that have been used in the past are normal saline

(Tauzuke *et al*, 1966; Cleveland *et al*, 1964), Ringer's lactate (Pifarré *et al*, 1967), dextran (Manax *et al*, 1964; Mundth *et al*, 1965; Makin and Howard, 1965; Ackermann and Barnard, 1966; Ackermann *et al*, 1966; Ringdal *et al*, 1967; Khastagir *et al*, 1968; Harmann and Turcotte, 1969), dimethylsulfoxide (Rudolf and Mandel, 1967), glycerol (Halasz *et al*, 1967; Pryor *et al*, 1971), plasma (Belzer *et al*, 1967, 1968, 1970; Belzer and Kountz, 1970; Belzer, 1971; Humphries *et al*, 1968 a & b; Scott *et al*, 1969, 1971; Løkkegaard *et al*, 1970; Benjamin and Gell, 1971; Alexander *et al*, 1970), anticoagulated blood (Humphries *et al*, 1964, 1967, 1968 a & b; Basso *et al*, 1967; Hendry *et al*, 1968; Lepkowski *et al*, 1970) and physiological balanced salt solutions (Manax *et al*, 1965 a & b; Collins *et al*, 1969, 1971 a & b).

Out of all the previous work, have evolved two basic schools of thought. These are pulsatile, versus non-pulsatile perfusion and storage methods. Chief representatives of these diversified views are Belzer (Belzer *et al*, 1967) and Collins (Collins *et al*, 1969) respectively. Belzer is of the opinion that continuous, hypothermic, pulsatile perfusion with cryoprecipitated plasma in the LI-400 preservation unit best fulfils the criteria of an ideal storage method. Collins thinks that brief, hypothermic, non-pulsatile perfusion with a solution mimicking intracellular solution, followed by hypothermic storage best fulfils these criteria.

Belzer *et al* (1967) described a method of extracorporeal perfusion of the kidney using filtered plasma, a pulsatile pump, a membrane oxygenator, and hypothermia at 10 degrees Centigrade. Extensive

experience with successful canine kidney preservation was followed by the successful preservation of a human cadaver kidney prior to transplantation (Belzer *et al*, 1968). A detailed description of his method was published in 1968 (Belzer *et al*, 1968). Prior to Belzer's work, kidney perfusion was carried out with a continuous, non-pulsatile flow. The main problem of prolonged, non-pulsatile perfusion was that of gradually increasing vascular resistance (Mandelbaum *et al*, 1965; Nakayama *et al*, 1963). A pulsatile pump was developed and the results were somewhat improved. Even in kidneys perfused with a membrane oxygenator and a pulsatile pump, preservation was not optimal. There was still a rise in perfusion pressure and a fall in flow would gradually develop that suggested some vascular obstruction. Conventional microscopic studies revealed no evidence of thrombi. However, fat stains showed multiple small emboli in the renal arterioles and renal tubules. It was postulated that the increasing vascular resistance and consequent rising perfusion pressure was caused by blockage of the vessels by lipid components that were liberated into the perfusate by denaturation. Soluble lipoproteins are readily damaged by conditions that are usually hazardous to plasma proteins, such as extremes of pH, changes of temperature, and exposure of interphases such as fluid-gas or fluid-glass. In Belzer's experiments, he decided to denature the lipoproteins deliberately in order to eliminate and prevent fat emboli. This was done by rapidly thawing the plasma from a temperature of minus twenty degrees Centigrade, whereupon the lipoprotein precipitated and this flocculent deposit could be removed by microfiltration. The removal of these lipids eliminated the problem of rising perfusion pressure

during preservation.

Plasma obtained from the blood bank usually has a sodium content of 170 mEq/L (milliequivalents per liter). For this reason the plasma is adjusted, by the addition of distilled water, to a sodium content of 144 mEq/L, a potassium content of 4 mEq/L, and a chloride content of 100 mEq/L. On a largely empirical basis, to each liter of plasma is added 4 mEq of magnesium sulfate, 250 mgms of dextrose, 88 units of insulin, 200,000 units of penicillin and 100 mgms of hydrocortisone. The perfusate is thawed, prefiltered through a coarse filter, and finally passed through the millipore unit where the smallest pore size is 0.22 microns. Belzer states that his experiences with plasma substitutes have been unrewarding. With these substitutes, perfusion appeared to be adequate but the viability of the organ was greatly impaired after reimplantation and contralateral nephrectomy. The plasma remained superior because of an undefined factor or factors. Belzer and associates (Belzer *et al*, 1970; Belzer and Kountz, 1970; Magnusson and Kiser, 1971; Kiser *et al*, 1971) have conclusively shown that prolonged successful human kidney preservation can reliably be achieved, utilizing hypothermia and pulsatile perfusion with cryoprecipitated plasma.

The actual mode of action of Collins solution has not been determined. Factors of importance probably include vasodilatation produced by procaine and magnesium, the preservative properties of magnesium, hyperosmolarity which might reduce anoxic cell swelling, intracellular composition reducing ionic exchange across the cell membrane and the presence of phosphate buffer. When he applied his

solution to human cadaveric kidneys, he modified its composition (Collins *et al*, 1971*b*). Phenoxybenzamine, a component of the previously described solution was not included because it caused turbidity. He showed in dog experiments that there was no difference between this solution (C_3) and the previous one, which included phenoxybenzamine (C_4) (Collins *et al*, 1971a). Smith *et al* (1971) and Belzer and Kountz (1970) have previously described the rationale for use of phenoxybenzamine. Linke *et al* (1972) did not use phenoxybenzamine because it was difficult to get into aqueous solution and because of concern expressed by the manufacturer about its stability and duration of effectiveness. He substituted methylprednisolone. Løkkegaard *et al* (1971) agreed with the composition of Collins solution, with the exception that he substituted papaverine for phenoxybenzamine, because it is more soluble. Watkins *et al* (1971) confirmed the work of Collins, but his data indicate that four of the additives are unnecessary (i.e. procaine, heparin, magnesium and phenoxybenzamine). Chapman (1971) reproduced Collins work. In addition, he obtained evidence that magnesium need not be present in the high concentration suggested by Collins. A reduction in the concentration of magnesium by one-sixth, made it possible to eliminate the precipitate which normally forms during the making of Collins solution. Liu *et al* (1971) confirmed the efficacy of Collins solution but was not able to improve it. Sharzer and Lawton (1972) found Collins solution to be significantly better than other solutions.

Not all authors have found that Collins technique is adequate. Woods *et al* (1971) obtained good results using Collins technique but continuous hypothermic pulsatile perfusion was better. Scott *et al*

(1971) criticized the method of procurement of the dog kidney before preservation experiments. He states it is unrealistic to remove the experimental kidney under ideal conditions (hydrated dog, diuretics used, etc.) and then to compare this to the human cadaveric donor situation where, in the majority of instances, the ideal circumstances do not exist. Eilert *et al* (1971) concurs with this criticism and does not avoid hypotension, or use mannitol diuresis, or other factors emphasized by Collins so as to better mimic the clinical cadaver procurement situation. Using as little as fifteen minutes warm ischemia, Scott was unable to achieve good results with Collins technique. He did achieve good results under the same circumstances utilizing the techniques of Belzer. Johnson *et al* (1972) included a warm ischemia time in his surgical protocol and compared the techniques of Belzer and Collins. Fifteen minutes or less of warm ischemia produced equally good results with Collins and Belzer techniques. From thirty to sixty minutes warm ischemia produced poor Collins results, but good Belzer results. In kidneys subjected to clinically realistic periods of warm ischemia, Moberg *et al* (1971) found good preservation only if hypothermic pulsatile perfusion was used. Sinha *et al* (1971) compared Collins and Belzer techniques in forty dogs and obtain superior results with the latter. Calne *et al* (1972) also concurs that with Collins technique, it is essential that there be no warm ischemic damage to the kidney and that Belzer's technique is successful even in ischemically damaged kidneys.

B. FORMULATION OF THE PROBLEM AND OBJECTIVES

The two most publicized kidney preservation methods in the recent lieterature are those of Belzer and Collins. Belzer's technique consists of an initial non-pulsatile flush of the donor kidney with a four degree Centigrade cooled solution of Ringer's lactate plus additives. This is followed by continuous, hypothermic, pulsatile perfusion on a Belzer LI-400 apparatus. Collins technique consists of a non-pulsatile flush of the donor kidney with a freshly prepared, four degree Centigrade cooled solution of intracellular composition, followed by storage in iced saline for the duration of the preservation.

The efficacy of both methods has been shown by many authors. There is, however, little information in the literature to indicate the tolerance of kidneys to mixed preservation methods. Woods *et al* (1971) subjected eight canine kidneys to four hours of continuous pulsatile perfusion, followed by storage for twenty hours in iced saline slush. After autotransplantation, and immediate contralateral nephrectomy, only two of their animals survived and had good renal function at long-term follow-up.

Scott *et al* (1971) subjected three canine kidneys to eighteen hours of continuous pulsatile perfusion, followed by six hours of storage in iced saline. Following autotransplantation, all dogs died of uremia. Kauffman *et al* (1971) subjected four human kidneys to combined methods. All kidneys survived and functioned after transplantation into the recipients, with one patient requiring post-operative dialysis for acute tubular necrosis. Renal artery and vein thrombosis

were the two most important causes of failure reported by Woods (1971). He postulated that perhaps intimal damage was accentuated by combining preservation methods.

Since starting this project, two additional authors have published data on combined techniques. Sterling *et al* (1971) reported data on thirty-four cadaveric kidneys which were preserved for up to 50.5 hours, utilizing a combined method of simple hypothermic storage, followed by pulsatile perfusion preservation. Immediate function was obtained in fifteen kidneys, or forty-four percent of those transplanted. Twenty-eight of thirty-four kidneys eventually achieved satisfactory function, two underwent hyperacute rejection, three demonstrated rejection during the period of acute tubular necrosis, and one perfused with a hyperosmolar perfusate, sustained cortical and tubular necrosis.

Santiago-Delpin *et al* (1972) observed that initial lavage with Collins solution, followed by hypothermic storage, gave worse kidney survival than pulsatile perfusion methods. Six hours of Collins preservation, followed by eighteen hours of pulsatile perfusion, also gave worse survival than continuous perfusion preservation alone.

In spite of the fact that there is little published data to indicate to what degree kidneys can withstand combined preservation methods, uncontrolled combination techniques continue to be used in the University Hospital and in other kidney sharing programs. At the University Hospital, Edmonton, a small number of cadaver kidneys have been received from across Canada in iced saline, and placed on the Belzer apparatus prior to transplantation. Also, kidneys have been removed from the Belzer at the University Hospital, and shipped else-

where in iced saline. The numbers have been too small to draw any conclusions regarding the wisdom of these practices.

The experiments which comprise this thesis were carried out with the following major objectives.

1. To indicate whether a Collins solution flush, followed by simple hypothermic storage in iced saline preceding and following continuous hypothermic pulsatile perfusion, has a significant effect on the immediate function of the autotransplanted kidney.
2. To determine if there is a particular combination of preservation methods which produces irreversible kidney damage.
3. To determine if there is a significant difference in the quality of kidney preservation achieved in different combinations of these preservation methods.
4. To determine if there is a demonstrable light or electron microscopic difference between the preservation groups chosen.

CHAPTER II

A. METHODOLOGY

In spite of the fact that experimental kidney transplantation has been performed in the Surgical-Medical Research Institute for fifteen years, the author could not find an adequate explanation of transplant techniques. For this reason, the methodology was written and illustrated in some detail.

Early on Day One, frozen pooled dog plasma was rapidly thawed (Belzer *et al*, 1967). After appropriate additives (Table 1) the perfusate was millipore filtered through a special apparatus (Fig. 1) to a pore size of 0.22 microns. The Belzer LI-400 (Fig. 2), with a separate head for animal experimentation was assembled and primed with perfusate. After cryoprecipitation and millipore-filtration, perfusate was always crystal clear (Fig. 3). The basic perfusion circuit is shown in Figure 4.

To allow the Belzer to always be accessible to the human transplant program, the machine was kept in the hospital, approximately four blocks from the transplant research area (Surgical-Medical Research Institute). As the Belzer preserves two kidneys simultaneously, it was possible to do two preservation experiments per run.

Collins solution was prepared and cooled to four degrees Centigrade (Table 2). An adult mongrel dog less than three years old, and weighing between fifteen and thirty-five kilograms, was

BELZER SOLUTION

1.	Cryoprecipitated plasma	850 ml
2.	Distilled water	135 ml
3.	KCl	2 ml
4.	Mannitol (25%)	18 ml
5.	$NaHCO_3$	18 ml
6.	Penicillin G (500,000 μ/ml)	1 ml
7.	Decadron	2 ml
8.	Insulin	120 units
9.	$MgSO_4$	8 mEq
10.	PSP	2 vials

TABLE 1

Figure 1 Millipore-filter system, to filter cryoprecipitated plasma

Figure 2 The Belzer LI-400 Apparatus

Figure 3, Cryoprecipitated, pooled, millipore filtered, dog plasma

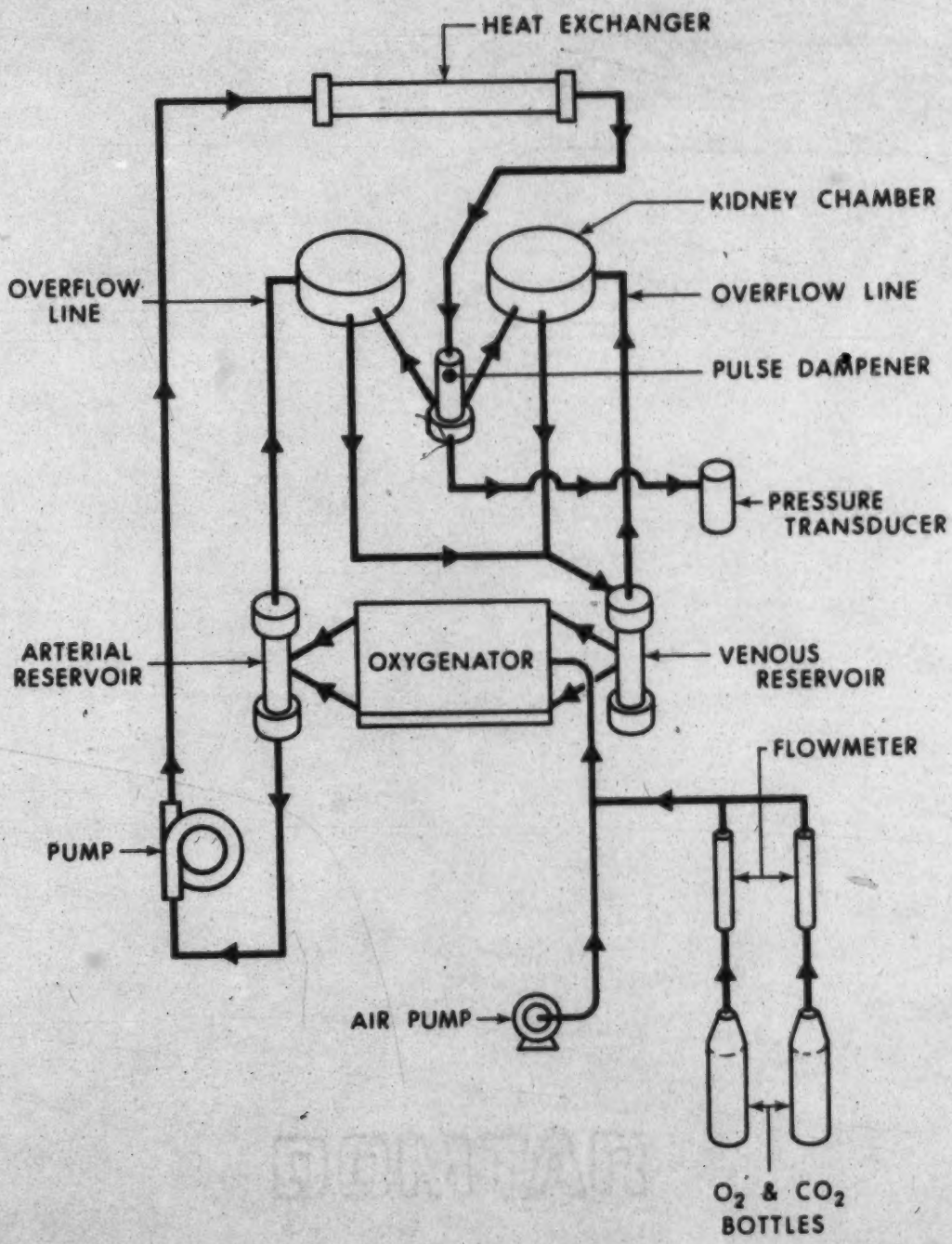

Figure 4. Perfusate circulation system (schematic)

COLLINS SOLUTION (C_3)

1.	KH_2PO_4	2.05 gm/liter
2.	$K_2HPO_4 \cdot 3H_2O$	9.70 gm/liter
3.	KCl	1.12 gm/liter
4.	$NaHCO_3$	0.84 gm/liter
5.	Heparin	5000 units/liter
6.	Glucose (50% w/v)	50 ml/liter
7.	Procaine (10%)	10 ml/liter
8.	$MgSO_4 \cdot 7H_2O$ (50% w/v)	14.4 ml/liter

TABLE 2

taken to the operating theatre, and tranquilized with acepromazine maleate. General anesthesia was induced using halothane by mask, and gentle restraint. The animal was intubated, and maintained under halothane anesthesia for the duration of the operative procedure. After preparing the skin with Betadine, the animal was draped. To simulate more closely the cadaver kidney procurement situation in our human transplant program, no intravenous fluid, diuretic, heparin, or other pre-treatment was used prior to nephrectomy.

A midline incision was made from the xiphoid process to the base of the penis in male dogs, or to the level of the mid-abdomen in female dogs. A Balfour retractor was inserted, and the left kidney was exposed by reflecting the spleen and the descending colon (Fig. 5). The peritoneum covering the antero-medial surface of the kidney was incised for the entire length of the kidney. The kidney was mobilized with a combination of blunt finger dissection posteriorly, and scissor dissection laterally, through the lateral fold of the peritoneum attached to the kidney. Numerous small collateral vessels were always found entering the kidney in relation to this peritoneal fold. These could always be divided directly with very little loss of blood, but the larger vessels were clamped and ligated with 00 cotton ties. This was particularly important for those on the lateral border of the kidney to avoid troublesome hemorrhage from this area after transplantation.

The renal artery and vein were carefully cleared. Small branches were noted, doubly ligated with 00 cotton, and divided between the ties. Failure to tie these branches prior to division, usually resulted in a hematoma in the wall of the vessel. Adequate vessel

Figure 5 Exposed left kidney prior to surgical removal

length was obtained by freeing the renal artery to the level of the aorta, and the renal vein to the point where the left testicular or ovarian vein joined in.

If a double artery was found on the left side, the right kidney was explored. In our experience, double arteries were usually bilateral, and were present in about twenty percent of animals. It was also common to find that the artery bifurcated soon after leaving the aorta, and lack of care in dissection and tying of the artery, resulted in double vessels in these cases. Because the cost of experimental dogs was high, those having double arteries were not discarded. A double renal artery cannula was utilized for perfusion of these kidneys (Fig. 6).

Adequate ureteral length was obtained by bluntly freeing the ureter down to the region of the pelvic brim, care being taken not to compromise its blood supply. The ureter was ligated distally with 00 cotton, and divided just proximal to the tie (Fig. 7). The kidney was placed in a bowl of iced saline slush after 00 cotton ligation and division of the renal artery and vein respectively. The artery was cannulated, and approximately three hundred cubic centimeters of cooled Collins solution was flushed through (Fig. 8). The kidney was placed in a transport jar containing iced saline and surrounded by ice in a styrofoam cooler (Fig. 9). The cooler was taken by car to the hospital laboratory. On arrival there, the renal artery cannula was attached to the appropriate place in the perfusion circuit (Fig. 10).

During transport of the kidney, the author's surgical technician,

Figure 6 Double artery cannula in place as kidney perfused on Belzer apparatus

Figure 7 Cleared renal artery and vein and divided ureter

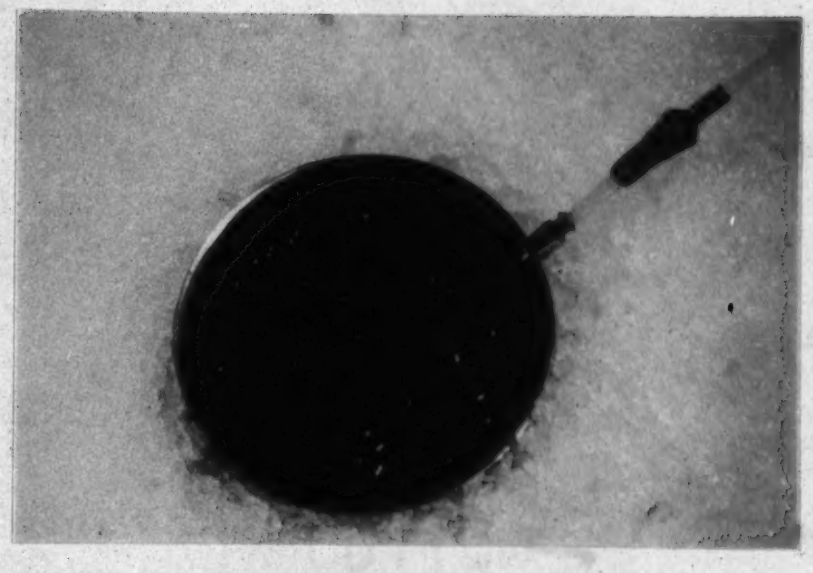

Figure 8 Left kidney in iced saline, being perfused with Collins solution

Figure 9 Transport jar surrounded by ice in cooler

Figure 10 Kidney in perfusion chamber of Belzer

Mr. Malcolm Wharton, remained with the animal to check for hemostasis in the nephrectomy bed, and to close the wound in layers. Linea alba was closed with a single continuous 00 chromic suture. Skin was closed with a subcuticular 0 chromic suture.

Immediately after placement of the kidney on the Belzer, the pulsatile pressure was adjusted to 60 millimeters of mercury. The pulse rate was adjusted to 60 beats per minute. The oxygen flow rate was adjusted to 0.5 liters per minute, and the carbon dioxide flow to 180 cubic centimeters per minute. The pH was checked occasionally during the remainder of the preservation, and kept close to 7.4 by adjusting the rate of flow of CO_2 gas through the membrane oxygenator. Depending to which group the dog had been randomly assigned, the kidney was now left on the Belzer for from five to twenty-two hours.

After completion of the Belzer portion of the preservation, the kidney was removed from the apparatus and flushed with approximately one hundred cubic centimeters of freshly prepared, cooled Collins solution. It was immersed in iced saline for the duration of the twenty-four hour preservation period. On the afternoon of Day One, a second kidney was similarly removed from another experimental animal, and subjected to the preservation procedures described above. After completion of the surgery, the animals were returned to the vivarium, given one bowl of soup, and water *ad libitum*.

On the morning of Day Two, the first dog was returned to the operating theatre, tranquilized with acepromazine maleate and anesthetized with halothane in a manner identical to Day One. After preparation of the skin with Betadine, the animal was draped, the midline

incision was reopened, and extended to one centimater above the pubis in both sexes. A Balfour retractor was inserted, and the right kidney was exposed by reflecting the ascending colon. The right renal artery, vein, and ureter were quickly exposed, and ligated with 00 cotton. The right nephrectomy was completed, the kidney was discarded, and hemostasis was achieved.

The transplant bed was now prepared. The lower abdomen was exposed by upwards retraction of the intestines behind saline soaked packs (Fig. 11). An incision was made in the posterior peritoneum distal to the aortic bifurcation, and medial to the right ureter. A retroperitoneal pouch was prepared by blunt finger dissection, tunnelling under the right ureter, and the peritoneum lateral to this point. The right common iliac artery and vein were cleared for a distance of several centimeters distal to the aortic and vena caval bifurcations (Fig. 12).

There was usually only one branch encountered during the clearing of the iliac artery, and it was always in an anterior location. Failure to ligate this prior to division, resulted in a hematoma in the wall of the vessel. During clearing of the iliac vein, a branch requiring division was seldom encountered laterally, but one was frequently located on the medial aspect of the common iliac vein, about one centimeter distal to the bifurcation. This was doubly ligated with 00 cotton when present, and divided between the ties.

At this point, the animal was heparinized with 300 milligrams per kilogram of heparin. The right common iliac artery was tied distally with 00 cotton, clamped well proximally with a bulldog clamp, and divided close to the tie. The common iliac vein was similarly

Figure 11 Right ureter crossing bifurcation of aorta

Figure 12 Cleared right common iliac artery and vein

tied distally, clamped proximally with a bulldog, and divided close to the tie (Fig. 13). The arterial and venous stumps were flushed with a small amount of normal saline.

The first two 6-0 silk sutures were placed in the stump of the common iliac vein. These were passed through the vein in an outside to inside fashion on the medial and lateral aspect. The preserved kidney was removed from the iced saline and the artery and vein were trimmed distally. The kidney was placed upside down compared to its anatomical location, and in the right iliac fossa. No further unnecessary delays were tolerated until completion of the arterial and venous anastomoses and release of the bulldog clamps, to make the period of warm ischemia as short as possible.

The two 6-0 silk sutures that had previously been placed in the common iliac vein, were now passed through the renal vein in an inside to outside fashion, and such that the renal vein would not be twisted when the sutures were tied (Fig. 14A). The nature of the above placement of sutures, also resulted in the knots being located on the outside of the veins.

A curved snap was applied to the short end of each suture to allow for traction and exposure of the anastomosis. The anterior half of the venous anastomosis was completed using a continuous running stitch (Fig. 14 B, C). This suture was tied to the short end of the second suture. The first suture was divided and the vein was inverted so that the second suture could be used to complete the posterior aspect of the venous anastomosis with a similar continuous running stitch (Fig. 14D, Fig. 15). The method described for the renal vein

Figure 13 Divided common iliac artery and vein; first two 6-0 silk sutures in vein

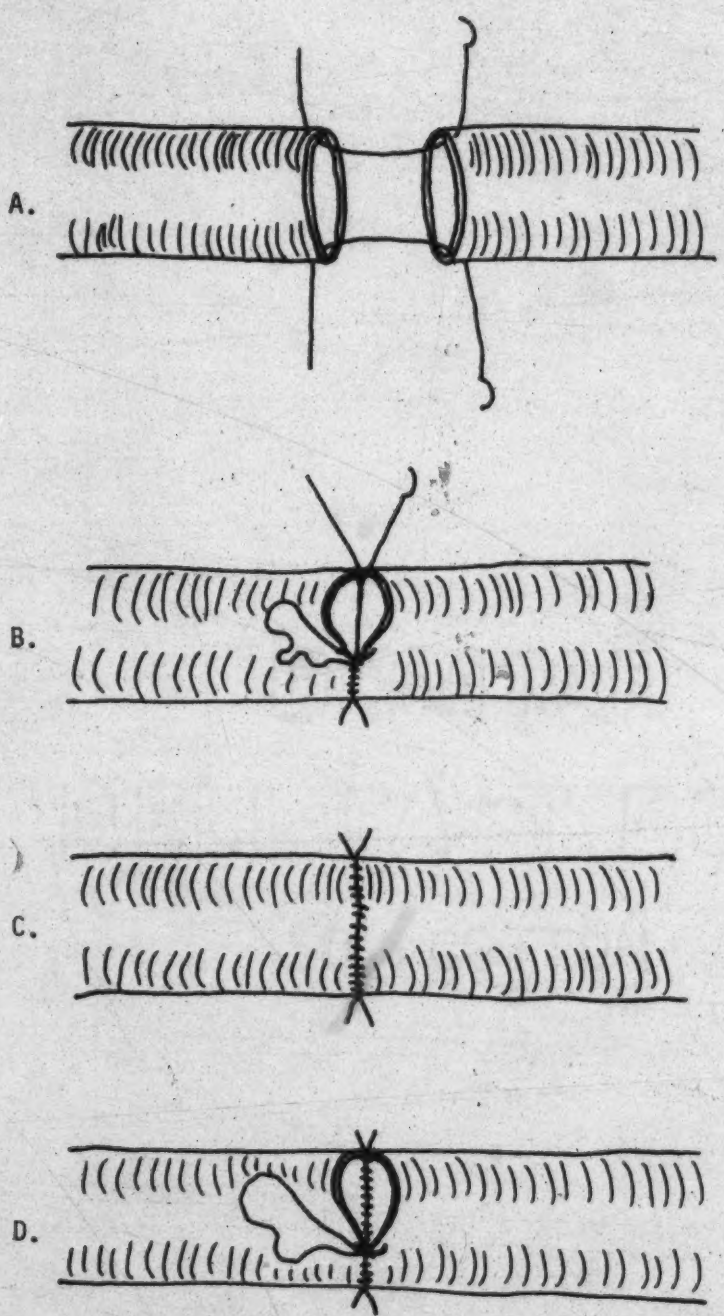

Figure 14. Technique of end-to-end vessel anastomosis.

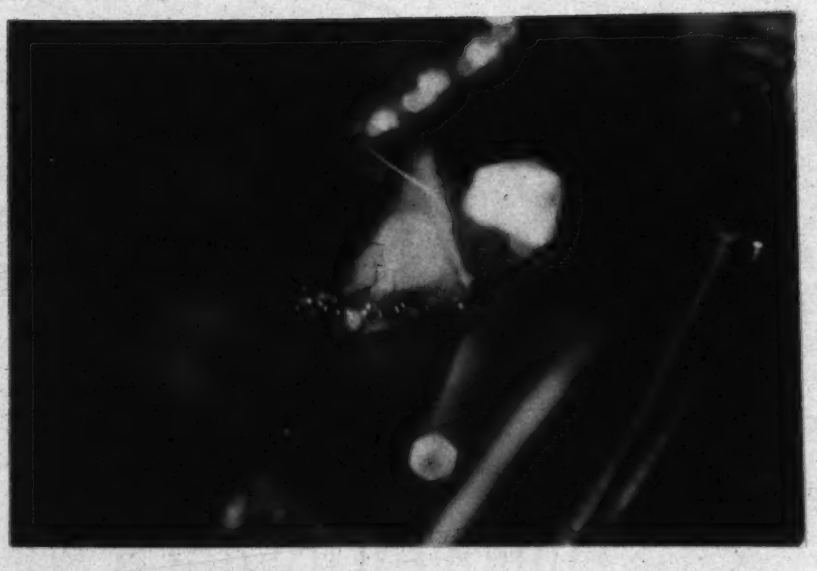

Figure 15 Completed end-to-end venous anastomosis

anastomosis was found to be the easiest to accomplish, but in many dogs an end-to-side anastomosis of renal vein to common iliac vein was fashioned using similar suture techniques (Fig. 16, Fig. 17).

The renal artery was anastomosed end-to-end to the common iliac artery using 6-0 silk, and suture techniques identical to those described for the venous anastomosis (Fig. 18). Immediately after completion of the arterial anastomosis, the bulldog clamps were removed from the vein and artery respectively. The renal artery was pinched lightly between two fingers, just proximal to the anastomosis to allow escape of air trapped in the vessel as the bulldog was released (Fig. 19).

During the transplant, the animal was hydrated by the administration of one liter of normal saline. After release of the bulldog clamps, the heparin effect was reversed by administration of 250 milliliters of 2/3:1/3 solution containing the appropriate number of milligrams of protamine sulfate. The solution also contained forty milliequivalents of furosemide to promote diuresis. After approximately fifteen minutes, to allow time for the protamine sulfate to reverse the heparin effect, the ureter was implanted into the bladder in the following manner. Usually within five minutes after release of the bulldog clamps, a good urine flow was seen coming from the ureter (Fig. 20). A three centimeter incision was made in the anterior aspect of the bladder using a scalpel. Residual urine was expelled from the bladder. Babcock clamps were applied to the cut edges of the bladder for retraction. A submucosal tunnel was made through the bladder on the trigone, and the ureter was pulled through it (Fig. 21). Redundant ureter was excised, and the end was spatulated using iris scissors.

Figure 16 Slit in side of common iliac vein to accommodate end-to-side anastomosis

Figure 17 Completed end-to-side anastomosis

Figure 18 Completed end-to-end arterial anastomosis

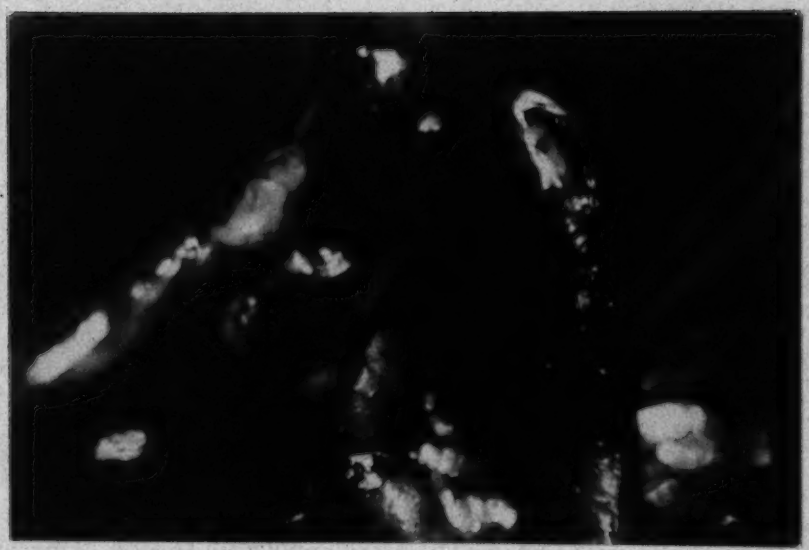

Figure 19 Appearance of anastomoses immediately after release of bulldog clamps

Figure 20 Ureter after release of the bulldogs with diuresis of urine occurring

Figure 21 Ureter pulled through sub-mucosal tunnel prior to trimming and spatulation

A probe was inserted into the ureter, and was left there during suturing to insure ureteral patency. Multiple 4-0 chromic, interrupted sutures were used to anchor the ureter to the lower end of the tunnel. The bladder was closed using two layers of 4-0 chromic suture. The kidney was placed in the retroperitoneal pouch, and the opening to this was narrowed with interrupted sutures.

Retractors and sponges were removed from the peritoneal cavity. The omentum was pulled down to its natural position. Linea alba was closed with a continuous 00 braided wire suture. Subcutaneous tissues were closed using continuous 00 chromic sutures. Skin was closed with a 4-0 braided wire subcuticular stitch.

In the post-operative period, renal function was followed with frequent serum creatinine (Owen *et al*, 1964), and blood urea nitrogen determinations (Fawcett *et al*, 1960). At six weeks post-transplant, a few kidneys were subjected to renal angiography and electron microscopy, and all kidneys were subjected to renal function studies (glomerular filtration rates and renal plasma flows) and light microscopy.

CHAPTER III

A. RESULTS

Seven groups of animals were included in the experimental protocol with animals being assigned to these in a random fashion. By the end of the experiment there were from four to eight animals in each group for a total of 43 long-term survivors. Thirteen animals were lost to technical difficulties in the operative or early post-operative period. Only long-term survivors will be discussed in the results.

Group 1 animals were the controls, and were subjected to donor nephrectomy, non-pulsatile flush with Collins solution, and immediate autotransplantation. In Groups 2 through 7, the kidneys were preserved for a progressively higher percentage of the twenty-four hour preservation period, by Collins technique, until in Group 7, Collins technique alone was used (Table 3). The poorest overall survival was achieved in Group 7 where sixty-six percent of the animals survived six weeks post-transplant. In the rest of the groups, over ninety percent of the animals survived six weeks post-transplant.

Numerous problems were encountered as the experimental protocol was carried out:

1. Technical Difficulties

Thirteen animals were lost to technical failure. The most frequent cause of technical problems was the presence of double renal

Group	Number of Animals	Collins	Belzer	Collins	Collins Technique Percent of 24 hrs
1	6	0	0	0	0
2	8	1	22	1	8.3
3	5	1	20	3	16.7
4	4	1	17	6	29.2
5	5	1	11	12	54.1
6	7	1	5	18	79.3
7	8	24	0	0	100

TABLE 3. Shows the seven experimental groups, the number of animals in each group, time the kidney was subjected to Collins and Belzer techniques in each group, and the percentage of 24-hour preservation that was Collins technique.

arteries in the donor kidney. This accounted for approximately one half of the failures. Other problems included arterial cannula leakage on the Belzer, Collins perfusate precipitation, two poorly performed vessel anastomoses, two dehiscences, and one anesthetic death.

Double vessels were encountered in about twenty percent of kidneys explored. The most sensible thing would have been to eliminate all with double vessels from the series. However, the cost of experimental animals dictated that these animals be utilized. About one half of the attempted double vessel anastomoses were lost to thrombosis. The other half performed normally and proved the value of continued use of these kidneys.

Two ways were utilized for dealing with these double vessels during anastomosis. Also, a double vessel cannula was required for perfusion of the donor kidney immediately after removal. The first suture method used was simply to place a single 6-0 silk suture to link the two arteries in the middle (Fig. 22). From this point onwards, the artery was treated as a single vessel, and anastomosed in an end-to-end fashion to the common iliac artery by the techniques described in the Methodology. This was found to be the best way of dealing with double arteries.

The second suture method used, consisted of spatulating the ends of the two renal arteries and suturing them together while the kidney was still immersed in iced saline (Fig. 23, Fig. 24). The artery was then anastomosed to the common iliac artery in the described manner.

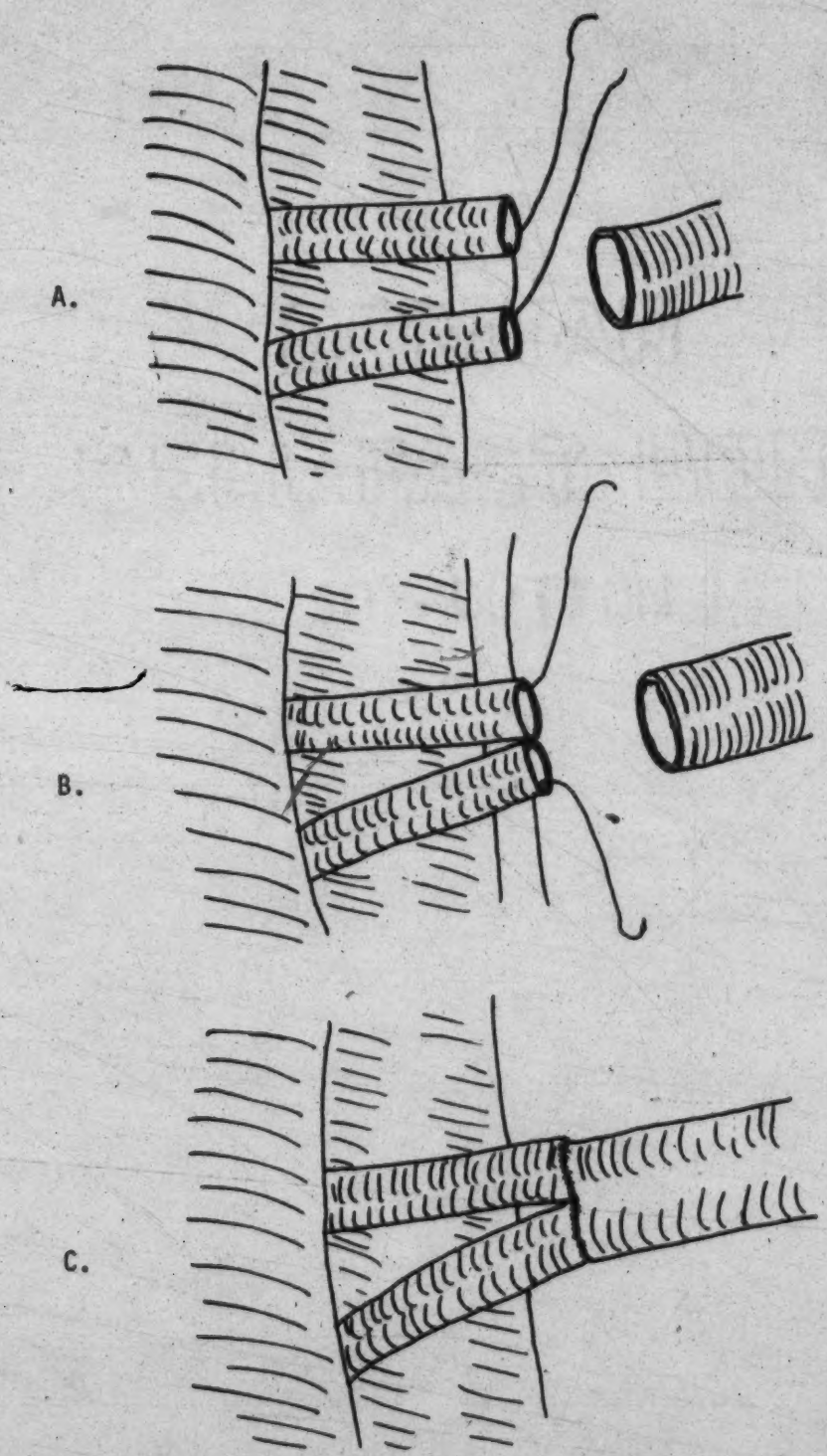

Figure 22. First technique of double vessel anastomosis.

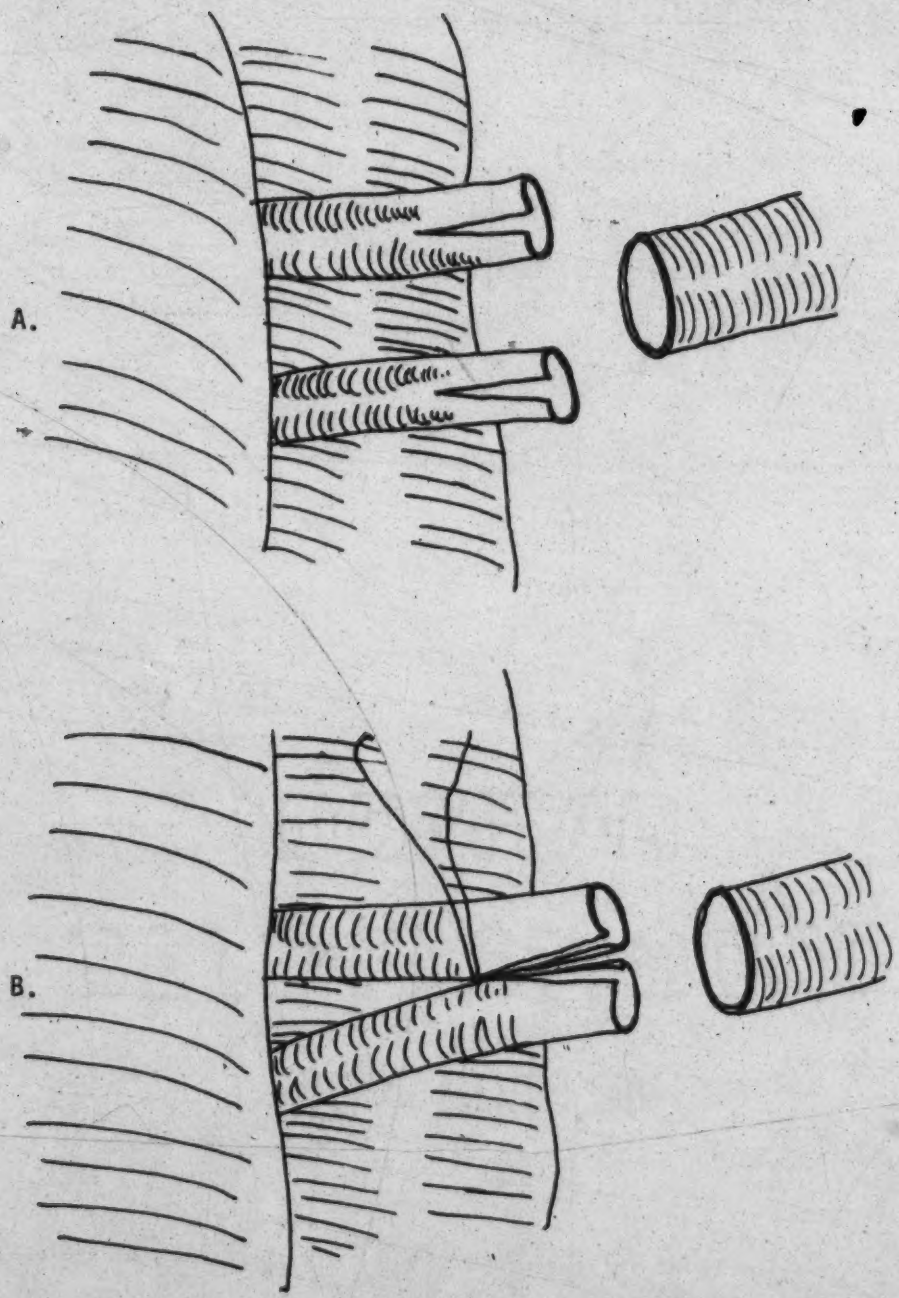

Figure 23. Second technique of double vessel anastomosis.

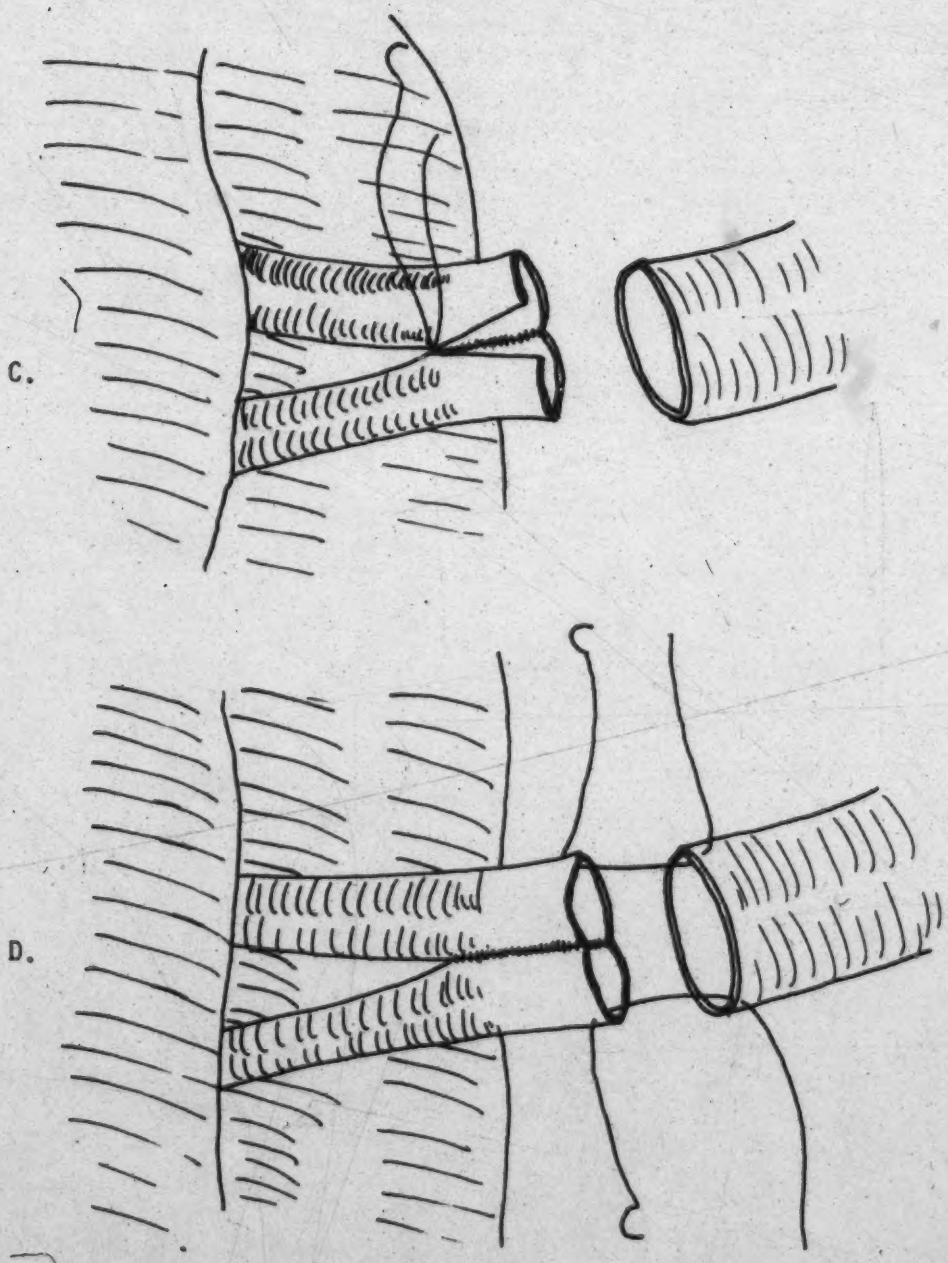

Figure 24. Completion of second technique of double vessel anastomosis.

2. Perfusate Problems

a. Collins solution

The most common difficulty encountered with this perfusate, was its tendency to precipitate soon after the additives were placed in the stock solution. Other authors have referred briefly in their papers to this problem, and relate it to the presence of phenoxybenzamine in C_4, and to the high magnesium content in both C_3 and C_4. The only way the author found to circumvent this, was to use the Collins solution within one to two hours after it was made. Through experience, it was learned that attempted use of precipitated Collins, resulted in poor perfusion of the donor organ initially, and eventually in the loss of the animal to renal failure.

b. Belzer solution

Shortage of dog plasma, and lack of cross-matching facilities at the research area, necessitated the use of pooled dog plasma. This opened the potential hazard of immunoglobulin deposition on the intima of the kidney during preservation. No untoward sequelae seemed to result from this.

3. The Belzer LI-400

As with any complex apparatus, considerable skill was required in the operation and maintenance of the Belzer. The two most difficult problems encountered, were related to machine design. The first, was the monitoring required to keep the pH in the normal range. Some competitive machines have been designed with automatic pH titrators, removing the necessity of frequent perfusate gas determinations. The

second problem, which was related to the first was the inadequate CO_2 gauge on the Belzer. A current of air was enough to alter the gauge setting, and hence CO_2 flow. This would result in a pH change. The only way the author dealt with the first problem, was through perfusate gas determinations, and corrective altering of machine settings to correct the pH. The only method found to deal with the second problem, was to elaborately tape the CO_2 gauge and try to reduce its sensitivity.

4. Wound Healing

The experimental protocol required entry into the dogs abdominal cavity on two consecutive days. It was found that with conventional suturing techniques, and using chromic suture, a wound dehiscence rate of one in fifteen ensued. Since it was obvious that any loss of experimental animals because of this was both wasteful and unnecessary, the author began closing wounds after the second operative procedure, with non-absorbable suture. Since this method of closure was adopted, there were no wound dehiscences in thirty consecutive transplants.

5. Vascular Thrombosis

It had previously been postulated that exposure of a kidney to two preservation procedures in the same twenty-four hour period would predispose the vessels to thrombosis (Woods *et al*, 1971). In the first twelve animals in the experiment, several developed renal arterial or venous thrombosis. The author felt that these thromboses were more likely related to the hazards of working with small vessels, where one can at best expect a patency rate of less than one hundred

percent. At this point, total body heparinization was introduced into the methodology, not unlike the technique used in human peripheral vascular surgery. Since inclusion of this step, no single renal arteries or veins have been lost to thrombosis, but some double renal arteries continued to be a problem.

6. Renal Vasoconstriction

It was known from the work of other researchers, that one could reduce the incidence and degree of vascoconstriction in the donor kidney, and thereby improve the quality of the subsequent preservation. Known ways of doing this included gentle handling of the kidney during nephrectomy, hydration of the experimental animal, mannitol diuresis, and administration of antivasospastic agents prior to donor nephrectomy. It was also known that many authors include these procedures in their preservation protocols. However, in human cadaver kidney procurement, such specialized procedures prior to nephrectomy, required early contact with the prospective donor. Since the latter was seldom realized in the University Hospital transplant program, it was deemed more practical to not pre-treat our experimental animals prior to donor nephrectomy. The nephrectomy was done, therefore, in an animal which was fasted but not fluid deprived. Any effects this method of procurement may have had on our results, is impossible to say, because no animals were done with donor pre-treatment for comparison.

B. RENAL ANGIOGRAPHY

It was hoped that some useful information could be obtained by comparison of the intrarenal vasculature in various groups as seen on

angiography. This goal was not fully accomplished, partly because of the lack of proper angiography equipment. The technique was limited to a flush aortic injection of contrast material, followed by the taking of a single x-ray (Fig. 25). It was determined early in the experiment, that the information obtainable under these circumstances, was not sufficient to justify the further spending of time and money, so this procedure was deleted from the protocol.

C. LIGHT MICROSCOPY

No significant differences were detectable between groups as compared by light microscopy. Several interesting observations were made, however, which occurred with equal frequency in all groups. The majority of the kidneys were normal in appearance (Fig. 26). Several showed patchy, cloudy swelling of the proximal convoluted tubules, and mainly in the juxta-medullary glomeruli (Fig. 27). In those kidneys so affected, the outer cortex was essentially spared. These were considered reversible changes in renal architecture.

Several kidneys showed evidence of mild hydronephrosis, through dilatation of Bowman's spaces and the collecting tubules (Fig. 28). This could be considered as evidence of ureteral obstruction or renal atrophy. It was known from experience with human kidneys subjected to ureteral re-implantation, that many undergo a reversible, slightly hydronephrotic stage in the post-operative period. This is related to edema around the lower ureter. It is conceivable that if the animals had been retained for a longer time period post-operatively, these hydronephrotic appearances would have reversed. The last light microscopic

Figure 25 Renal angiogram six weeks post-transplant

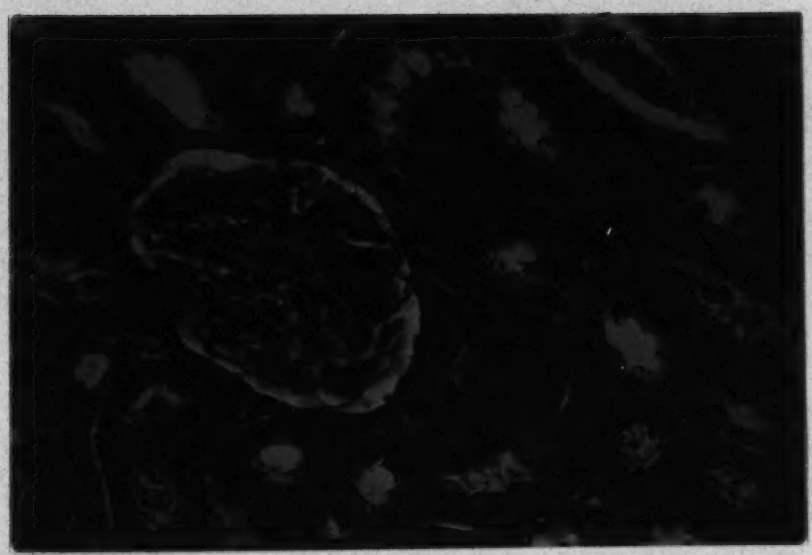

Figure 26 Normal light microscopic appearance of kidney

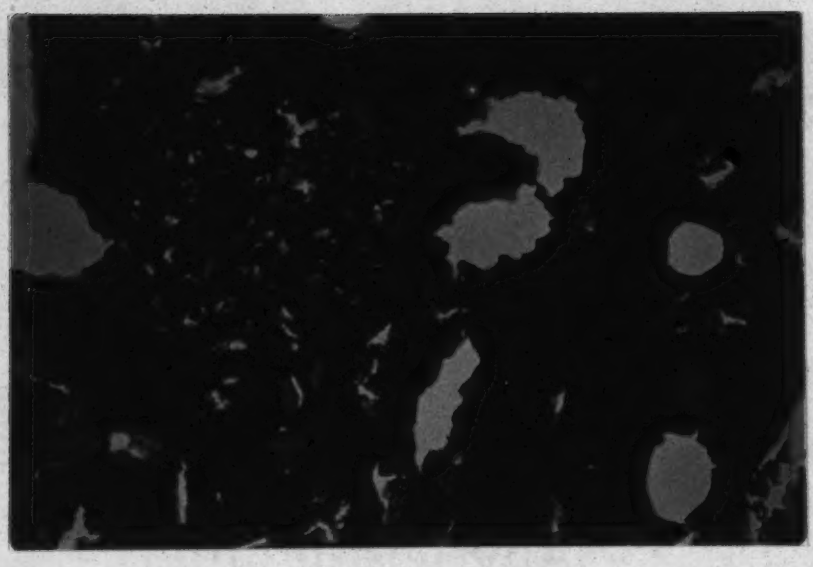

Figure 27 Juxta-medullary glomerulus showing cloudy swelling of proximal convoluted tubule

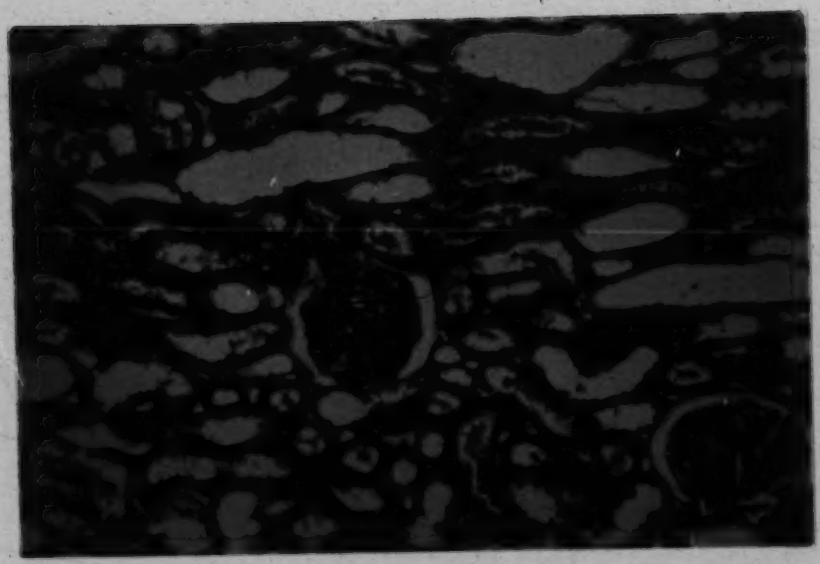

Figure 28 Mild hydronephrotic changes with dilatation of Bowman's spaces and collecting tubules

finding, was the occurrence of calcific deposits in some of the dilated collecting tubules (Fig. 29). The significance or reversibility of the latter was not determined.

D. ELECTRON MICROSCOPY

No significant differences were noted between groups by electron microscopy. Equal numbers of kidneys from each group were anlayzed until this conclusion became obvious. The electron microscopic appearances of all kidneys analyzed were essentially normal.

Mitochondria are known to be very sensitive indicators of anoxic injury to cells (Trump and Arstila, 1971). Most of the mitochondria observed were normal in appearance. A few showed minimal, but probably reversible swelling. In anoxia, which is often produced by ischemia, the initial interaction is to inhibit mitochondrial respiration and oxidative phosphorylation. This immediately stops much of the supply of Adenosine-triphosphate (ATP) to the cell and tends to increase the Adenosine-diphosphate over Adenosine-triphosphate ratio (ADP/ATP). In many cells, inhibition of mitochondrial respiration is followed by stimulation of anaerobic glycolysis, which may for a time sustain ATP levels. In most cells, however, this can occur only for a limited period; ATP supplies are rapidly depleted, since they are used for important homeostatic functions such as activities of the cell membrane. Changes in the ADP/ATP ratio, have immediate consequences for the mitochondria: matric granules are rapidly lost, and the matrix contracts severely. The contracted state of the mitochondria may be a very transient phase, since it is dependent on continued integrity of the mitochondrial inner membrane. When this

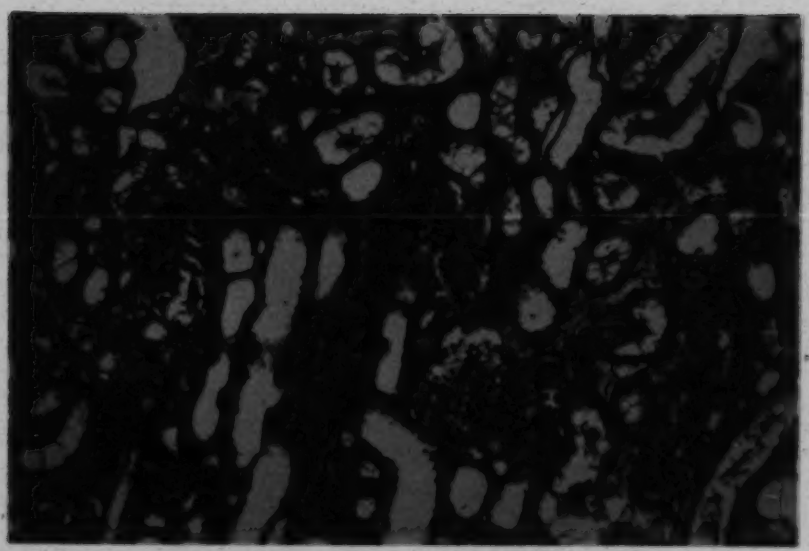

Figure 29 Calcific deposits in dilated collecting tubules

integrity is lost, high-amplitude swelling on the inner compartment ensues, probably augmented by the influx of calcium or other swelling agents through the damaged cell membrane (Fig. 30).

The decreasing supply of ATP, seriously affects cell membrane function. An early change, is a decrease in the rate of sodium extrusion, which tends to increase the sodium potassium ratio within the cell, and is accompanied by influx of cell water. This begins to produce cell swelling. This early change in cell volume seems to involve the cisternae of the endoplasmic reticulum which appear to form a reservoir for the early influx of sodium, chloride, and water. When severe, this produces a vacuolar change within the cell known as cloudy swelling which is visible by the light microscope (Fig. 27).

Cells can tolerate markedly decreased ATP levels for only a limited time which varies with the cell in question. Ultimately, the cell loses the ability to recover, even if the oxygen supply is restored. This is probably related to irreversible damage to the mitochondrial inner membrane, and closely follows the onset of high-amplitude swelling. Obviously, the minimal mitochondrial swelling shown in Figure 31, is not so-called high-amplitude swelling, and indicates a minimal degree of cell anoxic injury remaining at the time of analysis.

The remaining figures (Fig. 31, 32, 33 and 34) show normal electron microscopic appearances in a proximal, convoluted tubular cell, a distal convoluted tubular cell, and two glomeruli. Apart from occasional mitochondrial changes as illustrated (Fig. 31) and discussed in this section, no other electron microscopic changes were detected in the sections examined.

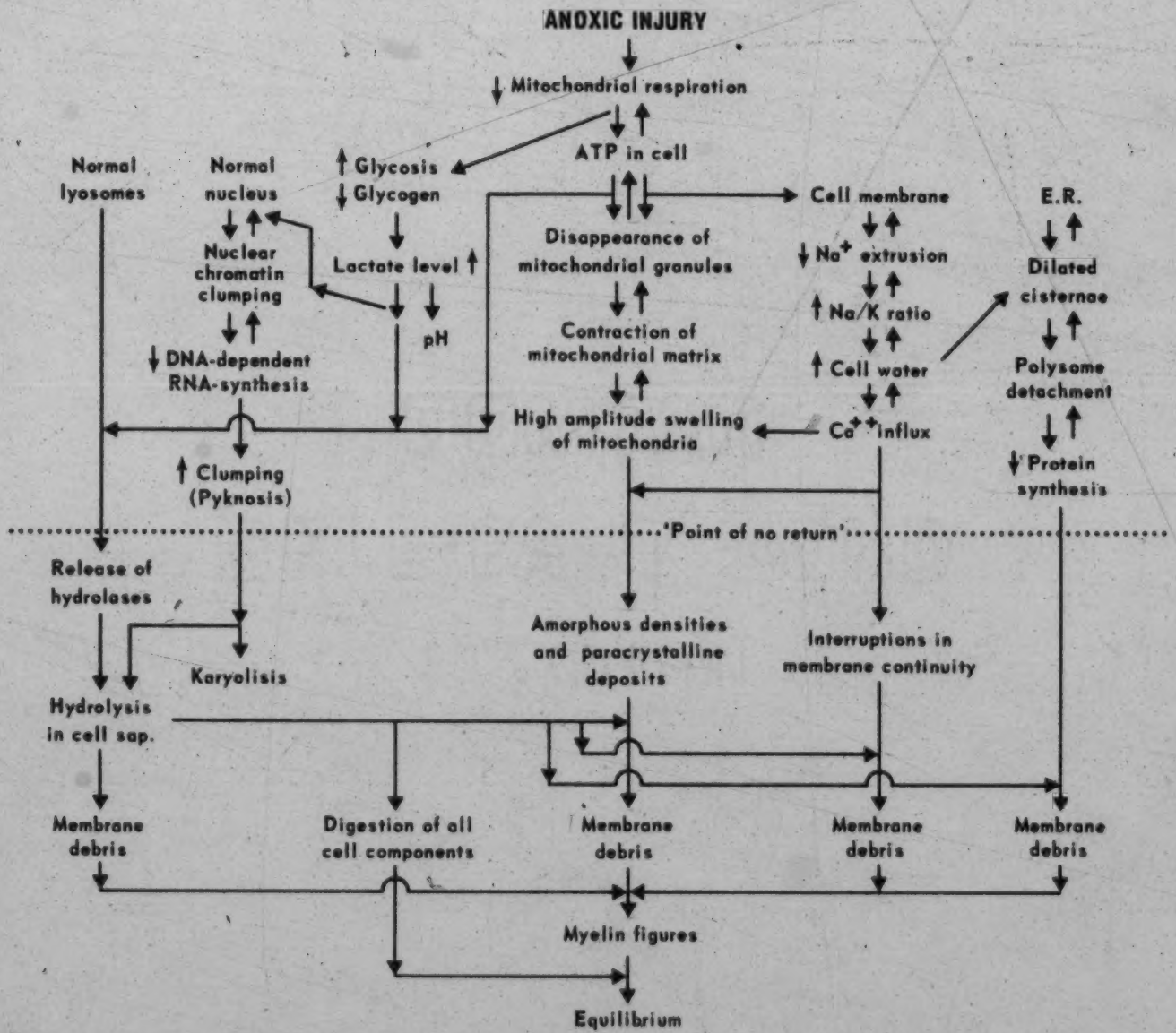

Figure 30. Diagramatic representation of the events in a cell after anoxic injury.

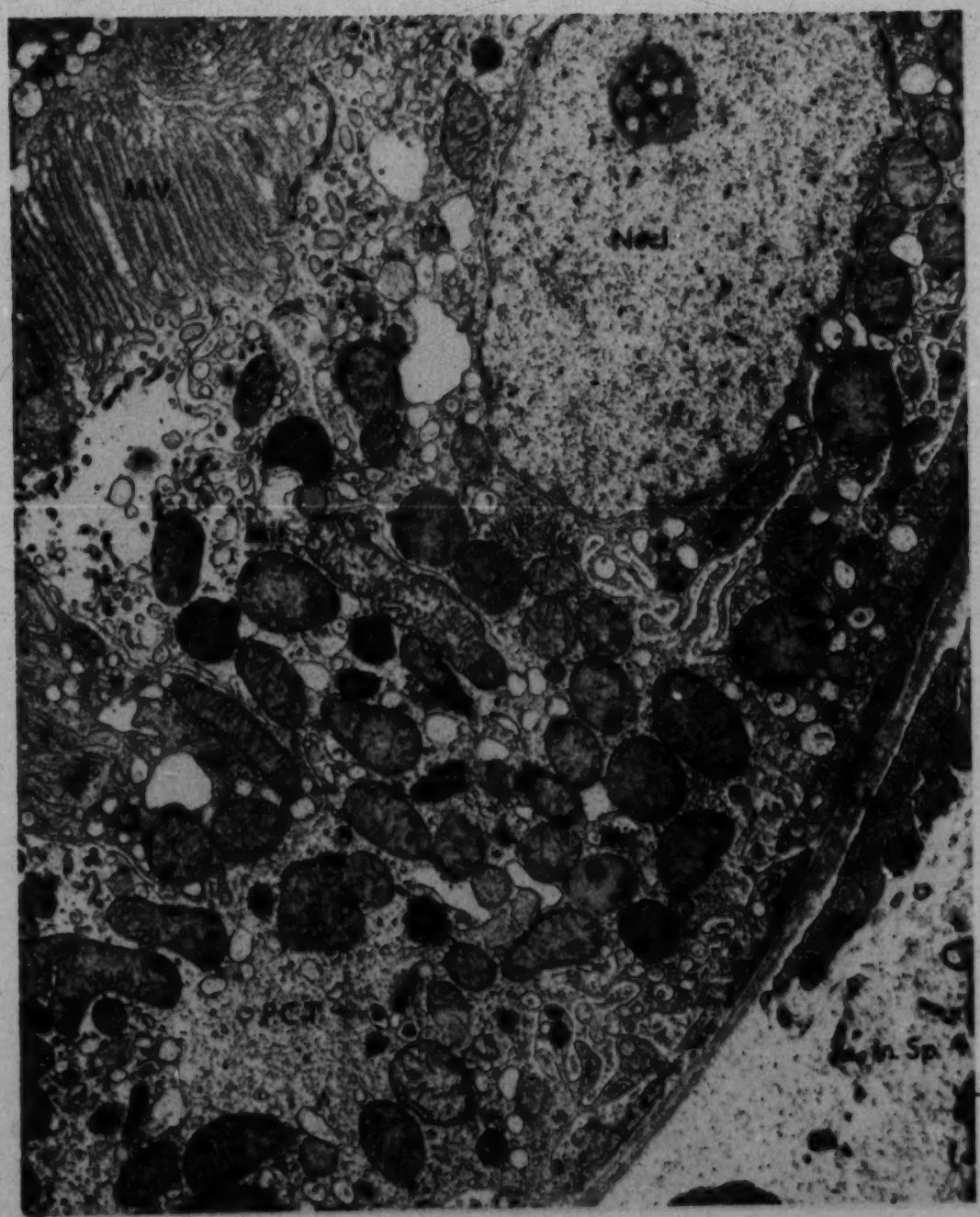

Figure 31 Electron microscopic appearance of proximal tubular cell six weeks post-transplant, showing microvilli (M.V.), nucleus (Nucl.), basement membrane (B.M.), interstitial space (In. Sp.), mitochondria (Mit.) and two arrows pointing to swollen mitochondria.

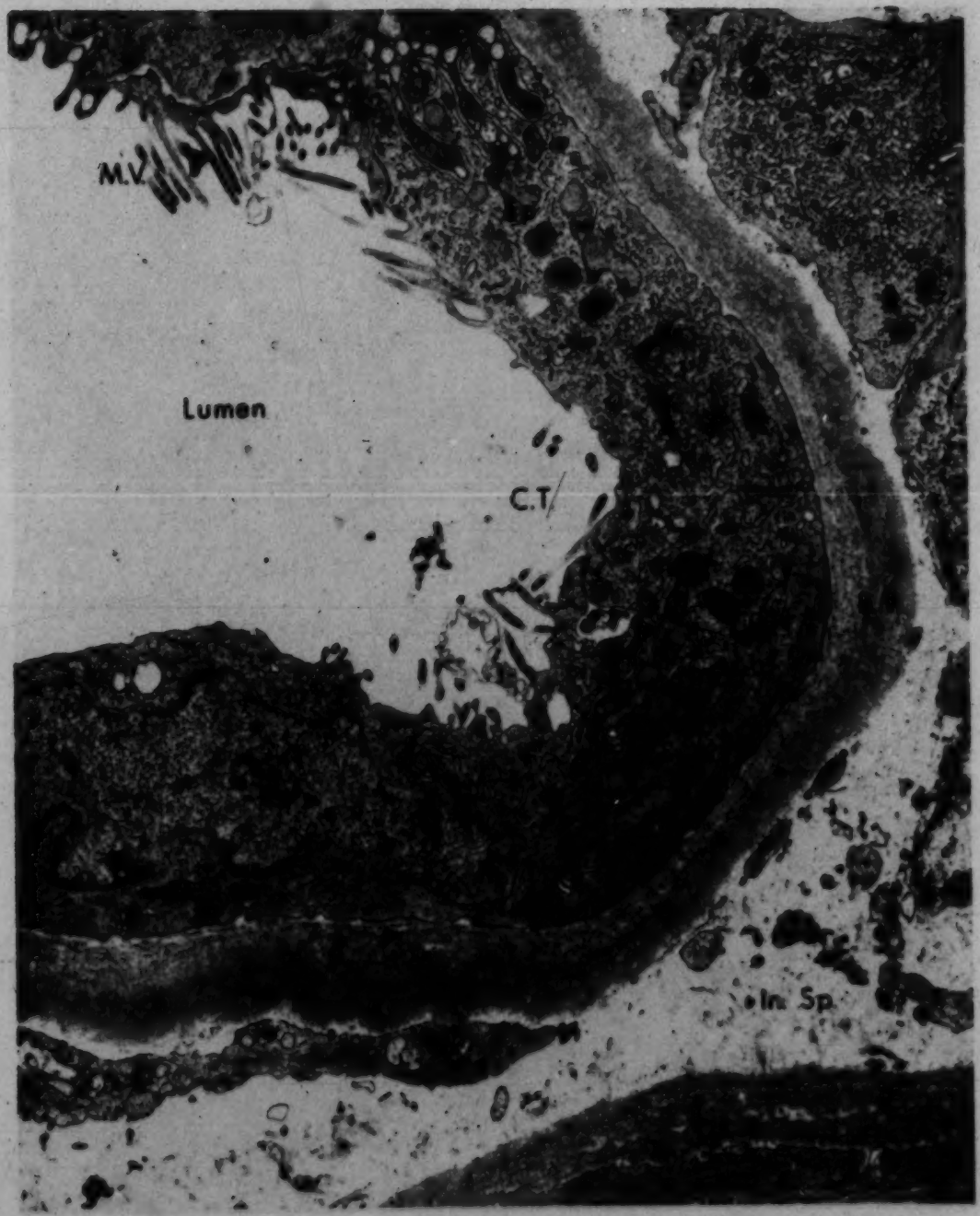

Figure 32 Normal electron microscopic appearance of distal convoluted tubular cell six weeks post-transplant showing microvilli (M.V.), basement membrane (B.M.) and epithelial cells (Ep.).

Figure 33 Electron microscopic appearance of normal glomerulus six weeks post-transplant showing foot processes (Ft. Proc.), basement membrane (B.M.), and epithelial cell (Ep.), a mesangial cell (Mes.) and a red blood cell (R.B.C.).

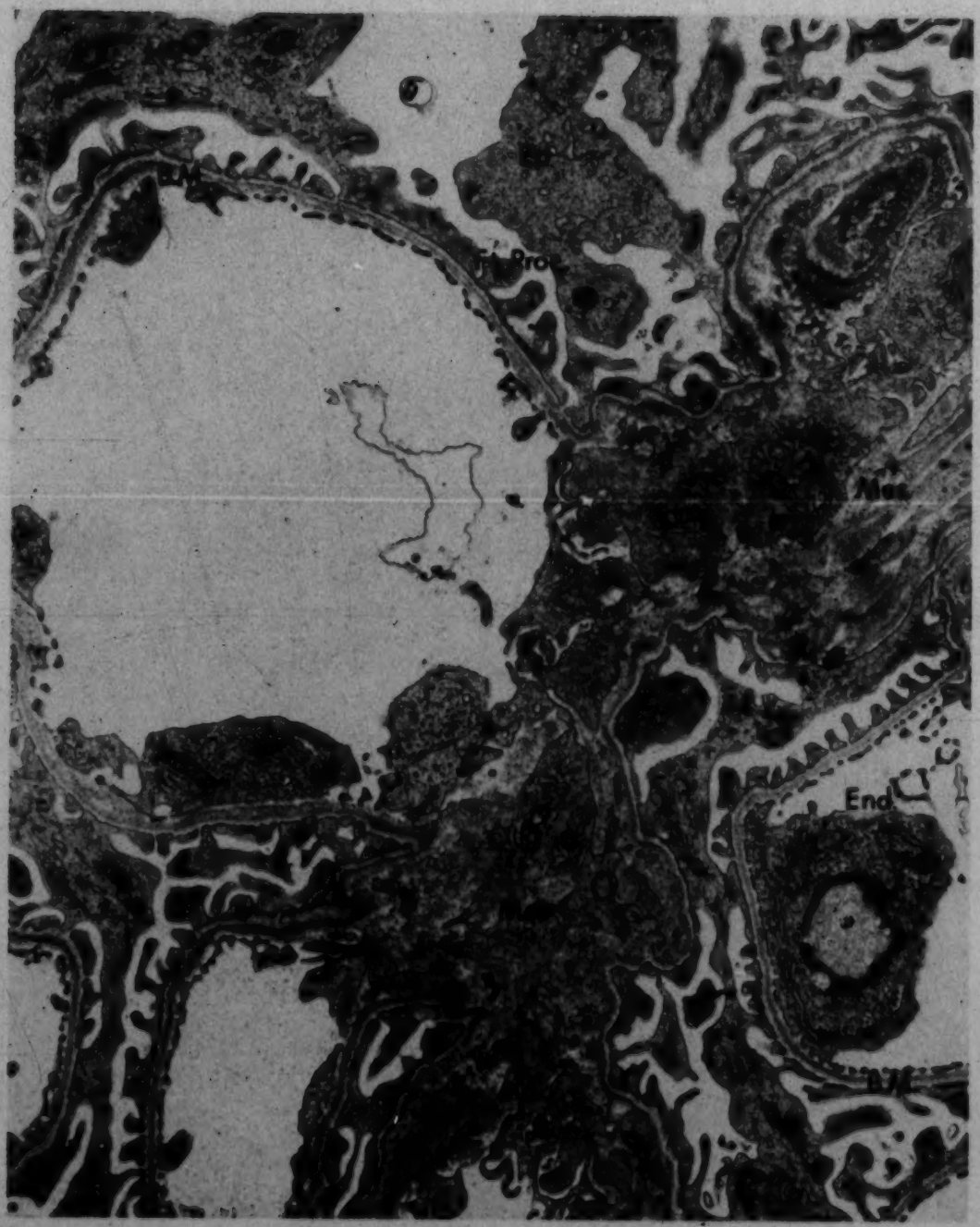

Figure 34 Electron microscopic appearance of normal glomerulus six weeks post-transplant showing basement membranes (B.M.), epithelial cell (Ep.), foot processes (Ft. Proc.), mesangial cells (Mes.) and an endothelial cell (End.).

E. CREATININE DATA

Serum creatinines for each animal were measured frequently in the post-transplant period. In the accompanying figure (Fig. 35), the creatinine data for four experimental groups is plotted against the days post-transplant. The data is plotted for days 0, 3, 7, and 42 post-transplant. Each point on the plots, is the average of the creatinine data from four to eight animals, depending on the group.

In all groups, except 7, the maximum average creatinine rise occurred on Day Three. The lowest post-operative rise, was in Group 1, or the control group. The next lowest rise, was in Group 2, where 22 hours of the 24-hour preservation period was on the Belzer. The highest creatinine rise, occurred in Group 7, where Collins technique alone was used. All other creatinine rises in other groups, fell between those plotted for Groups 2 and 7 (Fig. 35), with the plot from Group 4 being shown as representative of these groups. Average post-operative creatinine data and standard errors are shown for all groups (Table 4).

F. BLOOD UREA NITROGEN DATA

An exactly analogous relationship between groups to that described for creatinine, was found on analysis of post-operative blood urea nitrogen data. Lowest blood urea nitrogen rises, occurred in Group 1. Group 2 blood urea nitrogen rises, were the next lowest, and Group 7 blood urea nitrogen rises, were the highest. Blood urea nitrogen rises for the other groups, lay between those for Groups 2 and 7, with the plot for Group 4 being shown as representative of

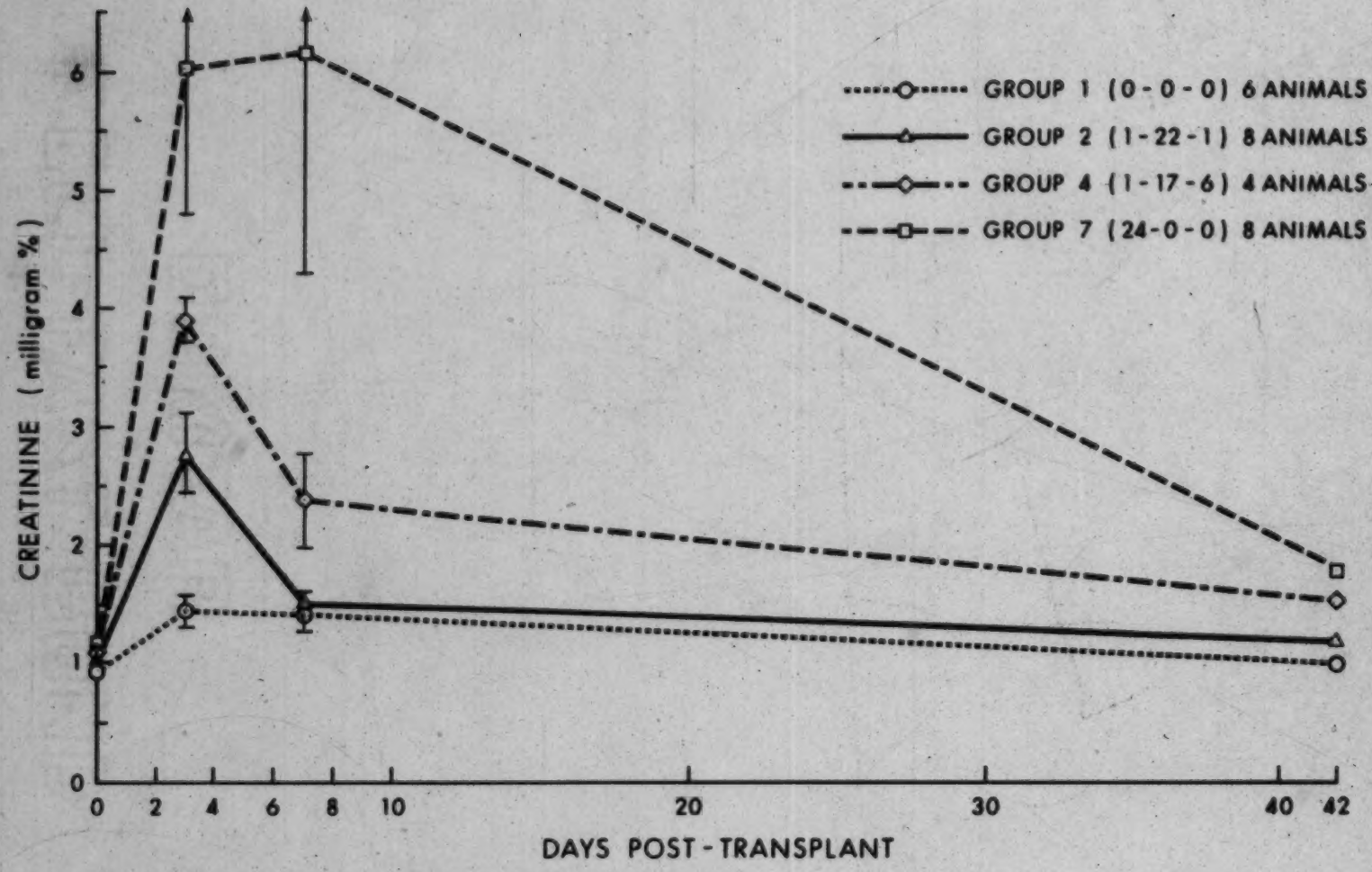

Figure 35. Post-transplant creatinine curves.

Group	Number of Animals	DAYS POST-TRANSPLANT			
		0	3	7	42
1	6	0.91±0.06	1.45±0.15	1.43±0.16	1.07±0.06
2	8	0.97±0.03	2.72±0.31	1.45±0.12	1.18±0.09
3	5	1.11±0.06	3.30±0.49	1.98±0.17	1.39±0.11
4	4	1.27±0.12	3.90±0.17	2.78±0.42	1.51±0.13
5	5	0.98±0.04	3.52±0.92	1.86±0.26	1.25±0.09
6	7	1.02±0.02	4.75±0.99	3.12±0.59	1.50±0.14
7	8	1.06±0.03	6.05±1.20	6.19±1.80	1.75±0.28

TABLE 4. Mean creatinines (milligrams percent) ± standard errors.

these groups (Fig. 36). Post-operative blood urea nitrogen data for all groups is shown (Table 5).

G. FRACTIONAL GLOMERULAR FILTRATION RATE DATA

The creatinine data can be analyzed in an additional way. If one subtracts the pre-operative creatinine, from the post-operative creatinine, on any particular day, one obtains a measure of the post-operative rise in the creatinine. It is well known that there is a constant relationship between the rise in creatinine and the glomerular filtration rate (GFR) in the so-called "steady state" (Kassirer, 1971).

The utility of the serum creatinine as an index of glomerular function, stems from the fact that any reduction in glomerular filtration, imposes a limitation on creatinine excretion; this impedence to excretion, in the face of a continued constant release of creatinine from muscle, leads to an accumulation of creatinine throughout total body water, and thus, to a rise in its serum concentration. Estimation of the extent of impairment of glomerular filtration from the concentration of creatinine, has as its basis the predictable relation that exists between a given level of GFR, and the creatinine concentration reached in the steady state. Thus, for every 50 percent reduction in GFR, the serum creatinine doubles. A patient with a normal serum creatinine concentration of 1 mg/100 ml, for example, has approximately 50 percent of normal glomerular function remaining when the serum creatinine has increased to 2 mg/100 ml, 25 percent of normal function remaining when the serum level is 4 mg, 12 percent at a serum creatinine of 8 mg, etc.

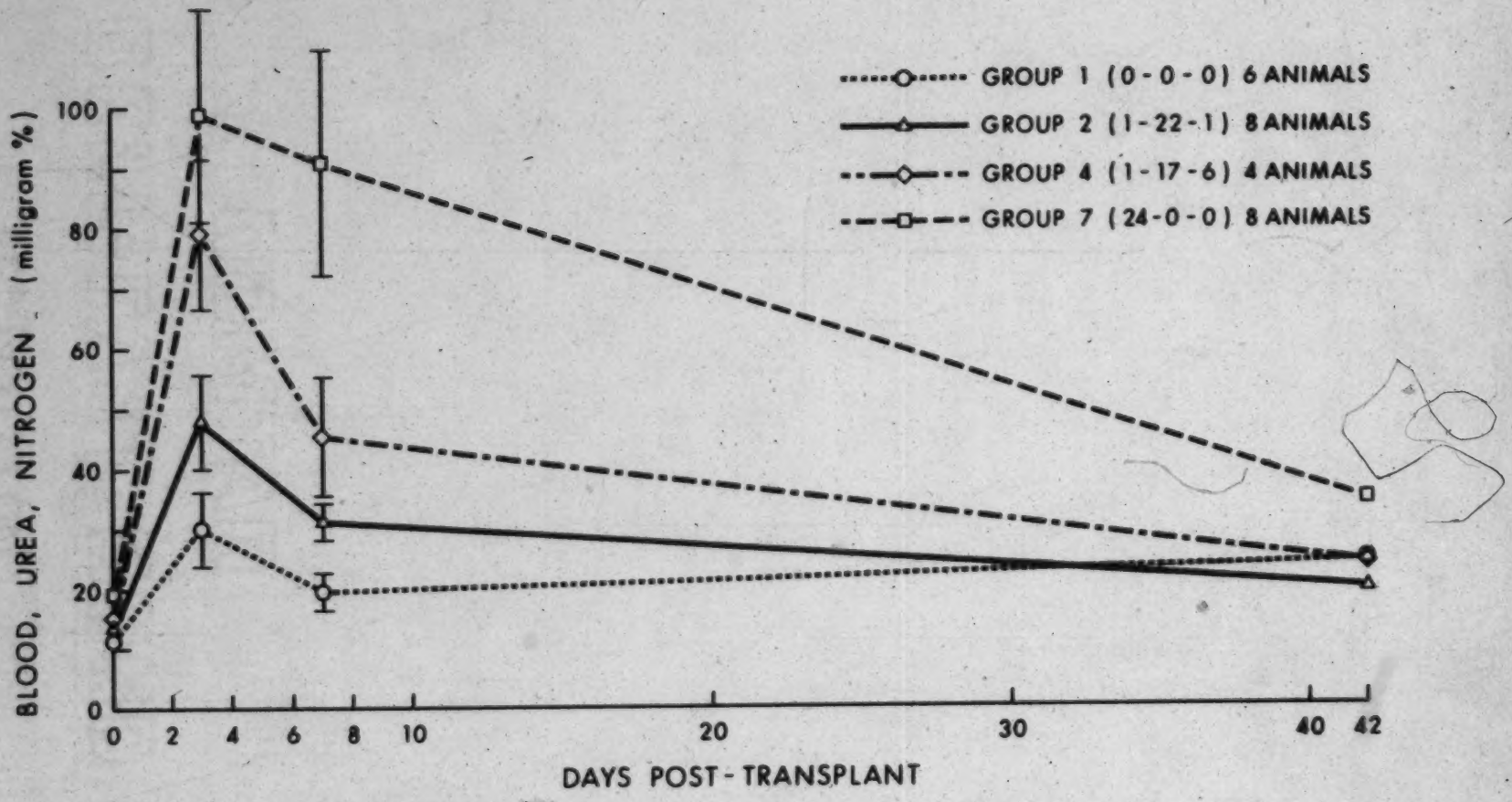

Figure 36. Post-transplant blood urea nitrogen curves.

Group	Number of Animals	DAYS POST-TRANSPLANT			
		0	3	7	42
1	6	11.5±1.3	30.0±6.2	19.9±3.0	24.2±1.2
2	8	14.9±1.1	48.1±7.4	31.0±2.7	19.9±2.6
3	5	16.3±4.5	70.7±15	34.3±5.5	23.3±2.3
4	4	18.7±5.5	79.3±12	45.2±9.3	23.8±0.5
5	5	16.5±2.3	67.9±17	35.5±7.4	17.8±2.9
6	7	13.8±1.7	91.7±16	58.5±17	26.9±3.0
7	8	13.2±1.5	98.6±17	90.6±18	34.3±5.9

TABLE 5. Mean blood urea nitrogens (milligrams percent) ± standard errors.

Another feature about the relation between the serum creatinine and GFR is worthy of emphasis: a small increase in creatinine concentration above the normal level, implies a much larger percent change in glomerular function, than the same absolute increase in serum creatinine when renal function is already moderately impaired. For example, an increase in serum creatinine from 1.0 to 2.0 mg/100 ml, implies a 50 percent decrease in function; an increase from 7.0 to 8.0 mg/100 ml, indicates only a 2 to 3 percent further loss of control GFR.

Using the above theoretical considerations, the creatinine data from all animals was analyzed. It was, thereby, possible to estimate from the serum creatinine, the fraction of the starting GFR remaining on any particular day (fractional GFR), the assumption being that the starting GFR was normal. The fractional GFRs so computed are listed in Table 6. Plots of the fractional GFR data for the groups, versus the days post-transplant, resulted in an exactly analogous relationship between groups to that reported in the discussion of the creatinine and blood urea nitrogen data above (Fig. 37). The lowest reductions in GFRs, occurred in the control group. The next lowest reductions, occurred in Group 2, and the greatest reductions occurred in Group 7. All GFR reductions for other groups, lay between the values reported for Groups 2 and 7. It can be seen from Table 6 that in Groups 1 and 2, the GFRs returned to about 85 percent of normal at six weeks post-transplant, whereas in Group 7 the GFRs returned to about 75 percent of normal. The trends of the data suggested that if the animals had been followed for longer than six weeks post-transplant, the absolute differences between groups might have been even less.

Group	Number of Animals	DAYS POST-TRANSPLANT			
		0	3	7	42
1	6	1.0	0.62±0.03	0.66±0.06	0.84±0.05
2	8	1.0	0.40±0.07	0.70±0.06	0.87±0.04
3	5	1.0	0.43±0.13	0.56±0.03	0.79±0.04
4	4	1.0	0.32±0.02	0.48±0.07	0.83±0.06
5	5	1.0	0.40±0.13	0.59±0.12	0.81±0.07
6	7	1.0	0.27±0.04	0.40±0.06	0.74±0.07
7	8	1.0	0.21±0.04	0.32±0.10	0.75±0.08

TABLE 6. Fraction of starting glomerular filtration rates remaining ± standard errors.

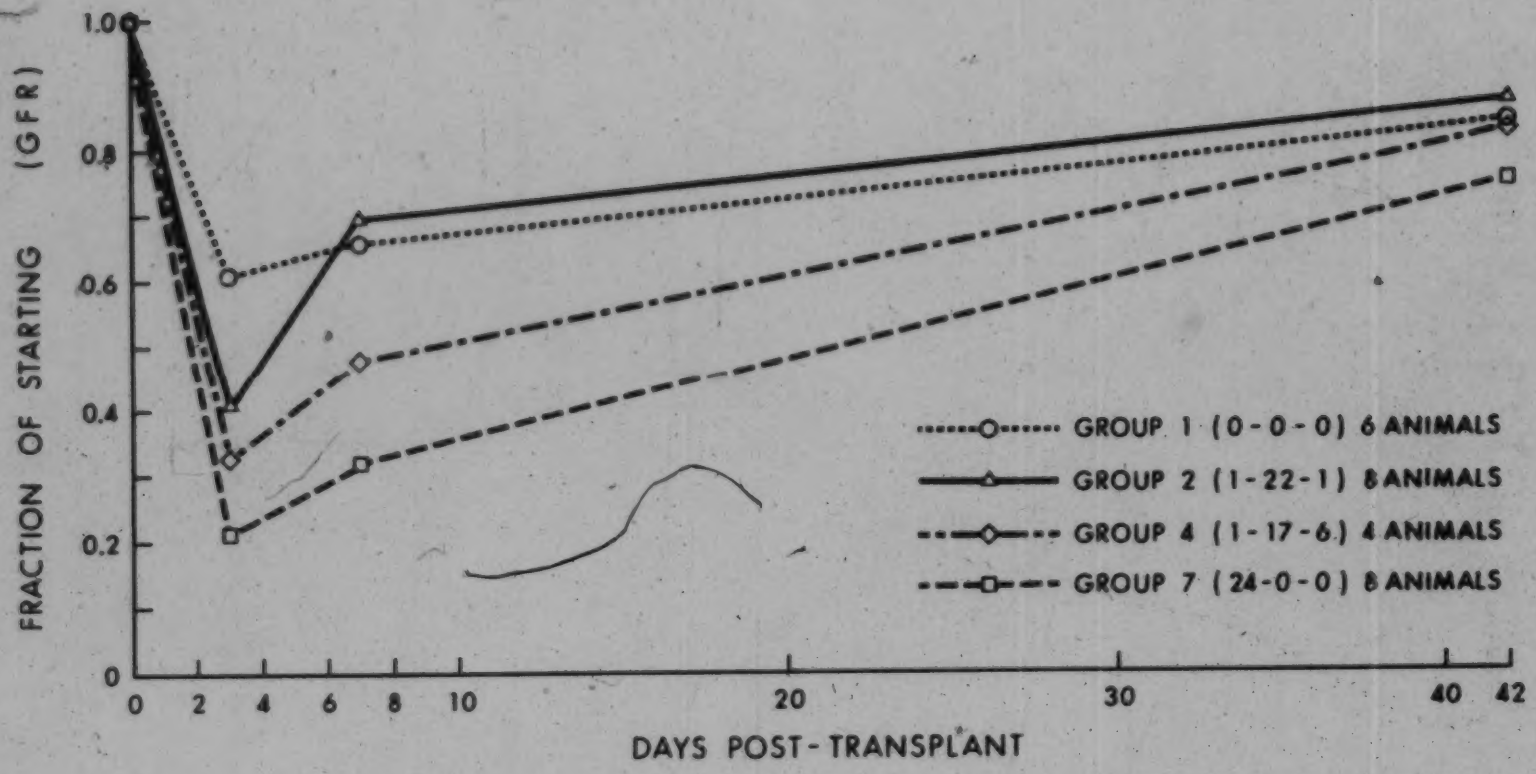

Figure 37. Fraction of the glomerular filtration rate remaining, versus day post-operative.

H. RENAL FUNCTION STUDIES

The values obtained for glomerular filtration rates (inulin clearances) and renal plasma flows (para-aminohippuric acid clearances) at six weeks are shown in Table 7. No predictable relationship was noted between groups. This was probably related to the fact that the studies were done at six weeks post-transplant, when the least differences were detectable between groups by even creatinine and blood urea nitrogen data. There was not enough technical help, or time available to perform these tests earlier in the post-operative period, when the data would probably have been more significant. The other uncontrollable factor was the wide variations in body weights between animals. An attempt was made to account for this by expressing the values per kilogram of body weight.

I. STATISTICAL ANALYSIS OF DATA

One might have wondered on looking at the data, if there was a progressive relationship between the data in different groups on a particular post-transplant day. In other words, was Group 2 data different from that of Group 1; was Group 3 data different from that of Group 2; was Group 4 data different from that of Group 3, etc., and were the differences progressive from group to group. The most logical way of checking this, was to subject the means of data on a particular post-transplant day, to a regression analysis. If there was a progressive relationship between groups, one would be able to draw a regression line through the plotted means, and with a high degree of significance.

Group	Number of Animals	GFR (ml/min/kg)	RPF (ml/min/kg)
1	6	1.82±0.14	5.50±0.55
2	8	1.92±0.14	4.23±0.40
3	5	1.52±0.15	3.90±0.36
4	4	1.22±0.23	4.10±0.47
5	5	1.60±0.18	6.27±1.10
6	7	1.60±0.21	4.41±0.50
7	8	1.71±0.26	3.37±0.74

TABLE 7. Renal function studies: Glomerular filtration rates (inulin clearances) ± standard errors; renal plasma flows (para-aminohippuric acid clearances) ± standard errors, performed at six weeks post-transplant.

For example, one could plot the mean creatinines of the seven groups on Day Three post-transplant, versus the percentage of the 24-hour preservation period the kidney was subjected to Collins technique (Fig. 38). The points on this plot were then subjected to a regression analysis, to see if a regression line could be drawn through the data. It was found that one could be drawn, with a significance at the 0.05 level or better.

A similarly significant progression between groups, was found on analyzing creatinine data at one week and six weeks post-transplant. In conclusion, this analysis indicated that as the percentage of time the kidneys were subjected to Collins technique was increased, the quality of the kidney preservation decreased in a progressive manner.

On analyzing the blood urea nitrogen data in a similar manner on days 3, 7 and 42, regression lines were once again applicable, in each instance, with significance at the 0.05 level or better. Hence the blood urea nitrogen data similarly indicated that as the percentage of time the kidney was subjected to Collins technique was increased, the quality of preservation decreased in a progressive fashion. Mean fractional GFR values were studied at 3, 7 and 42 days in an identical manner. Regression lines were once again obtainable in each instance with significance at the 0.05 level or better and the same conclusions as drawn from the creatinine and blood urea nitrogen data were obtainable.

Mean renal plasma flows and glomerular filtration rates as obtained from PAH clearances and inulin clearances respectively at six weeks post-transplant, were subjected to regression analyses. In neither case could a regression line be applied at the 0.05 level

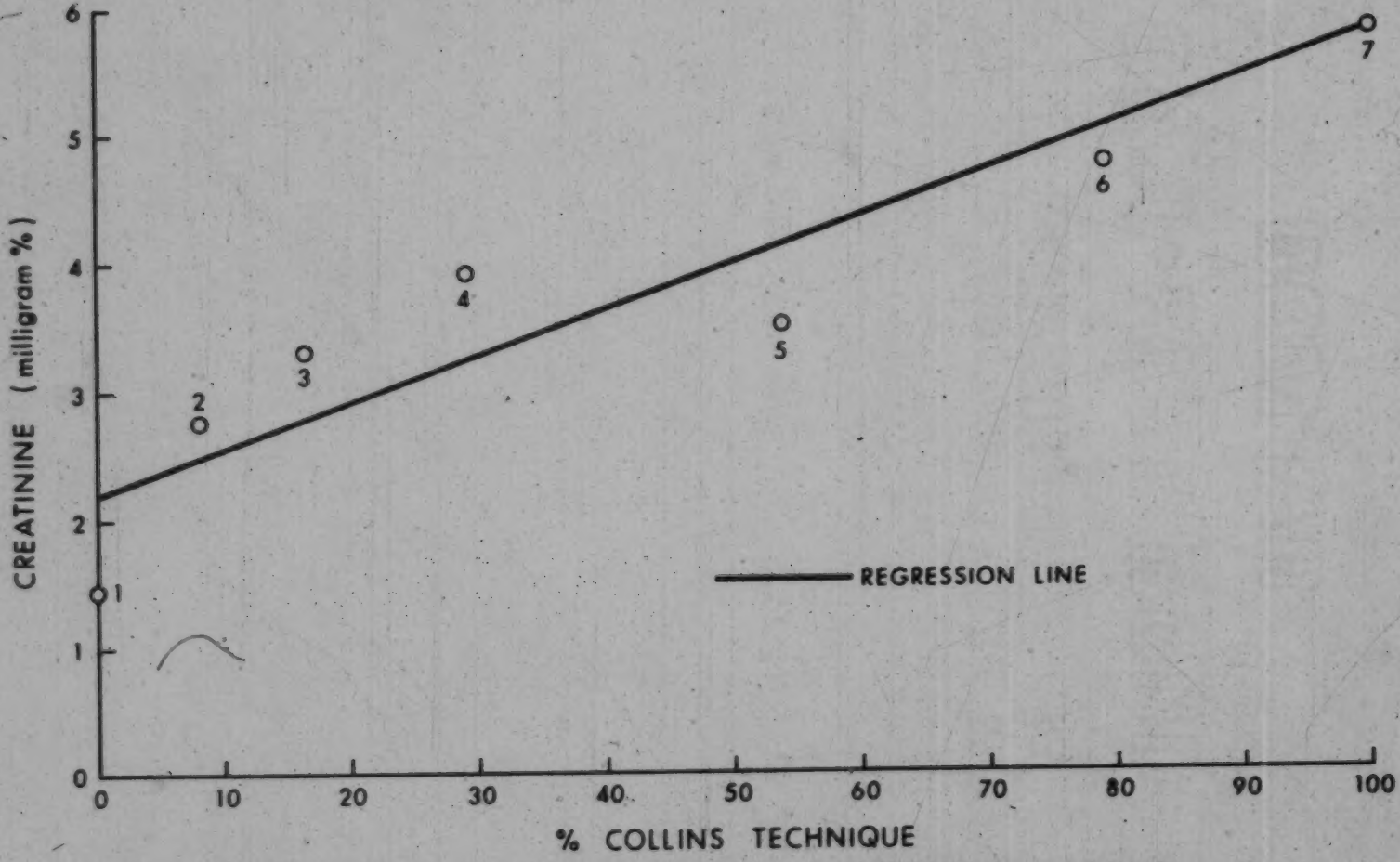

Figure 38. Mean creatinines at three days post-transplant plotted versus percent Collins technique for the seven groups. Regression line drawn which is significant at the 0.05 level or better, indicating a progressive relationship between groups.

Therefore, these renal function studies showed no progressive relationship between groups at six weeks.

CHAPTER IV

A. SUMMARY AND CONCLUSIONS

The primary aims of this thesis were to determine whether dog kidneys could survive subjection to combined kidney preservation techniques over a 24-hour period, to determine if significant differences in qualities of preservation would result from different combinations of Belzer and Collins techniques, and finally, to determine if there would be light or electron microscopic differences between preservation groups. The experimental animals were randomly assigned to seven groups. Group 1 animals were the surgical controls, with the left kidney being removed, flushed with Collins solution and immediately autotransplanted. In groups 2 through 7, the kidneys were all preserved for twenty-four hours. As the group number increased, the percentage of the twenty-four hour preservation that the kidney was subjected to Collins technique was increased, until in Group 7, Collins technique alone was used.

Approximately equal numbers of dogs were lost from all groups because of technical failure. After elimination of technical failures, the poorest overall survival rate was achieved in Group 7, with sixty-six percent of animals surviving six weeks post-transplant. In the rest of the groups, over ninety percent of animals survived six weeks post-transplant.

Analysis of post-transplant creatinine and blood urea nitrogen

data, showed that in most groups the maximum rises above preoperative values, occurred on the third post-transplant day. The lowest rises in creatinines and blood urea nitrogens, occurred in the control group. The next lowest rises were in Group 2, where twenty-two of the twenty-four hours preservation, was on the Belzer. The greatest rises, were noted in Group 7, where Collins preservation alone was used. The rises in creatinines and blood urea nitrogens for all other groups lay between those found for Groups 2 and 7. Analysis of fractional glomerular filtration rate data, indicated an identical relationship between groups.

Regression analyses were applied to the means of all data at 3, 7 and 42 days post-transplant. It was found that regression lines could be drawn through the means of data whether the unit of measure was creatinine, blood urea nitrogen, or fractional GFR and, with significance at the 0.05 level or better. This indicated that there was a progressive relationship between groups up to six weeks post-transplant, such that as the percentage of the Collins technique in the twenty-four hours was increased, the quality of preservation decreased. However, the absolute differences between the groups at six weeks, were small, and there were indications that if the animals had been followed for a longer time period post-transplant, the differences might have been even smaller. One can only speculate whether these relationships between groups, would be found if donor pre-treatment was used.

No significant differences between groups were detected by light microscopy, electron microscopy, renal angiography, or renal function studies done at six weeks post-transplant.

The conclusions which can be drawn from this study are:

1. Belzer and Collins techniques of kidney preservation can be combined successfully. It should be possible to take human kidneys off the Belzer, and ship them safely to other transplant centres.

2. The higher the percentage of the twenty-four hour preservation that Collins technique was used, the poorer was the quality of preservation. This relationship might have been different, if donor pre-treatment had been used.

3. To avoid the need for dialysis in the post-transplant period, and to avoid its associated increased morbidity and mortality, it is suggested by this study, that Belzer's kidney preservation technique should remain the preservation method of choice in the University Hospital, human transplant program.

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